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Topic Category Bacteria and Parasites: From Microbiome to Antibiotics

Abstract 1200

Polymyxin B resistance of *Vibrio vulnificus* is controlled by the CarRS two-component system

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Two-component system (TCS) plays critical roles in survival of enteropathogenic bacteria by sensing environmental changes and regulating the expression of genes involved in bacterial defense. One of the barriers that enteropathogenic bacteria encounter during host infection is cationic antimicrobial peptides (CAMPs), which bind to outer membrane of Gram-negative bacteria by electrostatic interaction and induce cell lysis. It is known that a fulminating human pathogen *Vibrio vulnificus* has intrinsic resistance to the CAMP polymyxin B (PMB). However, little is known about the TCSs of *V. vulnificus* responsible for the PMB resistance. In this study, we identified that the CarRS TCS, composed of sensor kinase CarS and its cognate response regulator CarR homologous to those of *Vibrio cholerae*, contributes to the PMB resistance. Transcriptome analysis revealed that CarR strongly activates the expression of the eptA operon, the tolCV2 operon, and carRS itself. Particularly, biochemical analyses identified that genes in the eptA operon are required for complete development of CarR-mediated PMB resistance. Additional research showed that CarS activates CarR by phosphorylation, leading to the activation of the eptA operon and the subsequent increase of the PMB resistance. Electrophoretic mobility shift assays and DNase I protection assays demonstrated that CarR directly binds to the consensus sequence in the upstream regions of the eptA operon and carRS itself. We further identified that CarRS senses PMB, leading to an alteration of the carRS expression. The combined results suggest that the CarRS TCS of *V. vulnificus* could contribute to the bacterial survival against the host-derived CAMPs during host infection.

This work was supported by the National Research Foundation of Korea, the Ministry of Science, Information and Communications Technology, and Future Planning (2021K1A3A1A20001134) and Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ016298), Rural Development Administration, Republic of Korea.

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Abstract 1223

High-throughput Virtual Screening of Potential Novel Inhibitors for DXP Reductoisomerase in *Helicobacter pylori*

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Josh Beckham

Helicobacter pylori infections are present in nearly half of the entire human population, with a significant proportion experiencing deleterious effects. DXP Reductoisomerase (DXR) is an integral enzyme in the isoprenoid biosynthesis pathway of many pathogenic microorganisms, including *H. pylori*. Since this is an essential metabolic pathway for organism viability, enzymes in the pathway are attractive for small molecule therapeutics. In order to identify potential inhibitory molecules, virtual screening of libraries of over a hundred thousand chemical compounds was carried out using the molecular docking softwares GOLD and ICM, against a homology model of HpDXR. The results of the screening showed that compounds ZINC−13 and ZINC−82 had fitness scores nearly double that of DXP, the native substrate. In order to validate the predictions from virtual screening, recombinant HpDXR protein was expressed via autoinduction and purified through nickel affinity chromatography and assayed for the effects of the ligands on its thermostability by differential scanning fluorimetry (DSF). Given these results from these preliminary screenings, the two compounds may show promise as potential inhibitors and will be tested using enzymatic assays in the near future. Novel compounds from this study may lead to new treatments for *H. pylori* infection.

We would like to give special thanks to the Freshman Research Initiative for their generous funding of the Virtual Drug Screening stream; without which, this research would not be possible.

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Abstract 1228

An Aniline-substituted Bile Salt Analog Protects Both Mice And Hamsters From Multiple *Clostridioides difficile* Strains

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Clostridioides difficile infection (CDI) is the major identifiable cause of antibiotic-associated diarrhea. The Centers for Disease Control and Prevention (CDC) reported that in 2017, over 223 900 people were diagnosed with CDI in the United States. In that same year, approximately 12 800 people died of CDI-related complications within 30 days of initial diagnosis. With an average of $35,000 to treat a single inpatient case, the cost burden to the U.S. health care system can reach between $3.2 billion and $4.8 billion annually. The emergence of hypervirulent *C. difficile* strains has led to increases in both hospital- and community-acquired CDI. Furthermore, the rate of CDI relapse from hypervirulent strains can reach up to 25%. Thus, standard treatments are rendered less effective, making new methods of prevention and treatment more critical. A key characteristic of *C. difficile* is its ability to form endospores (or spores). The spores' dormant nature allows them to survive in the gastrointestinal tract of susceptible patients. When the spores reach the nutrient-rich intestinal lumen, they can germinate into toxin-producing vegetative cells that cause symptomatic infection. Since spore germination is a necessary step for CDI establishment, methods that target this process could prevent infection. Previously, the bile salt analog CamSA (cholic acid substituted with m-aminosulfonic acid) was shown to inhibit spore germination *in vitro* and protect mice and hamsters from *C. difficile* strain 630. This present study shows that CamSA was less active in preventing spore germination by other *C. difficile* ribotypes, including the hypervirulent strain R20291. Thus, additional bile salt analogs were screened for *in vitro* germination inhibition activity against strain R20291, and the most active compounds were tested against other strains. An aniline-substituted bile salt analog, CaPA (cholic acid substituted with phenylamine), was found to be a better antigerminant than CamSA against eight different *C. difficile* strains. In addition, CaPA was capable of reducing, delaying, or preventing murine CDI signs with all strains tested. CaPA-treated mice showed no obvious toxicity and showed minor effects on their gut microbiome. CaPA's efficacy was further confirmed by its ability to prevent CDI in hamsters infected with strain 630. These data show promising results for the continued exploration of CaPA and other bile salt analogs to serve as potential prophylactic treatments in CDI-susceptible patients.

This research was supported by the National Institutes of Health (NIH) grant R01-AI109139, GM103440, and GR08954 and the National Aeronautics and Space Administration (NASA) Nevada Space Grant NNX15AI02H.

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Abstract 1252

Glucose and Mannose sugar analogs negatively affect KSHV replication by inhibiting N-Glycosylation and inducing the Unfolded Protein Response

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Kaposi's Sarcoma (KS) herpesvirus (KSHV) has been identified as one of the seven known human oncogenic viruses. KSHV infection precedes the development of KS, an AIDS-associated malignancy. While the incidence of AIDS-KS globally has seen a decline, this endothelial cancer is refractory to treatment and remains a significant burden to patients living with HIV. The standard of care for AIDS-KS has gone virtually unchanged for over 20 years and effective treatments against the oncovirus KSHV have yet to be developed. 2-DG (2-deoxy-D-glucose), 2-FDG (2-fluoro-deoxy-D-glucose), and 2-DFM (2-deoxy-2-fluoro-D-mannose) are sugar analogs. 2-DG and 2-FDG are D-glucose analogs and strong glycolysis inhibitors. 2-DG is also a D-mannose analog so that can inhibit N-glycosylation. Viral glycoproteins are mass produced, modified by N-glycosylation and folded in the ER and Golgi at the end of the lytic stage. In this work we aimed to characterize and compare the inhibition of the three drug analogs in infected iSLK.219 cells. We determined the ability of each drug to inhibit KSHV reactivation and virion infective capacity by flow cytometry using the KSHV producer cell line iSLK.219 harboring a recombinant virus (rKSHV.219) that contains an infection marker (GFP) and a reactivation marker (RFP). Virus titers were measured by de novo infection of naïve AdHEK293 cells with virus-containing supernatants from iSLK.219 cells and quantifying the number of AdHEK293 infected cells (GFP-positive). We assessed viral glycoprotein expression by western blot and quantifying virion production by qPCR. To confirm our hypothesis that N-glycosylation impairment is the main contributor of virus replication inhibition, we carried out D-mannose rescue experiments and analyzed activation of the unfolded protein response (UPR), which is triggered by ER stress following of N-glycosylation disruption. When UPR cannot be resolved, it leads to either apoptosis or autophagy. For this reason we analyzed the cellular fate of reactivated sugar analog-treated cells by western blot using apoptotic and autophagic markers. Additionally, we calculated the death index using the IncuCyte® machine, which allows for real-time cell quantification, with a Cytotox Red Reagent. Death index was calculated with the formula: Red object count (dead cells)/Green object count (cells). All three sugar analogs negatively affected KSHV replication and virion infectivity, but 2-DFM displayed the strongest inhibition. Although the number of reactivated iSLK.219 cells was reduced with the drug treatments, the most important inhibitory effect seems to take place at the late

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lytic stage. Viral glycoproteins K8.1 and gB were down-regulated and virion production was diminished in presence of the sugar analogs, especially with 2-FDG and 2-DFM. D-mannose could rescue viral glycoprotein expression and virion infectivity, but could not reverse the number of reactivated cells, indicating that N-glycosylation is the main target of the drugs. In line with this, all analogs triggered UPR and even overcame KSHV-induced suppression of the PERK downstream pathway. Finally, 2-DG, 2-FDG, and 2-DFM played a protective role in reactivated cells, displaying the autophagic marker instead of the apoptotic signature observed in the untreated cells. Collectively, this work identifies the non-toxic inhibition of N-glycosylation by sugar analogs as an important viral target and reinforces the potential of 2-DG as an antiviral for KSHV and other enveloped viruses. We also found that 2-DFM is a more potent and target-oriented compound and a promising therapeutic antiviral agent.

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Abstract 1253

Methylglyoxal, an active compound derived from monofloral manuka honey, produces bactericidal effects in *Salmonella typhimurium*

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Pamela Harvey

The rapid development of antibiotic resistance and decreased antibiotic development contribute to an urgent need for novel antibiotics. Historically, compounds isolated from natural products have been important sources of antibiotics. Methods of discovery have included compound screens and the identification of active compounds in natural products. We combined these approaches to screen the active compounds in manuka honey, a monofloral honey produced by bees that pollinate Leptospermum scoparium in New Zealand and Australia. In *Salmonella typhimurium* cultured in liquid media similar in composition to conditions in which the bacterium replicates *in vivo*, we found that compared to two other forms of honey, manuka honey limits bacterial growth, likely due to its relatively low sugar content. We then identified and tested each of the most abundant compounds in manuka honey using the agar diffusion test. Testing of fourteen compounds revealed that methylglyoxal (MGO) confers the greatest antimicrobial effects, and in subsequent experiments, demonstrated that MGO exhibits bactericidal effects on *Salmonella typhimurium*. Exploitation of natural products continues to represent a valuable strategy for identification of novel antimicrobials, and MGO represents a promising compound for antibiotic development.

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Abstract 1261

Development of T3SS Inhibiting Nanobodies

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Due to the immediate threat of antibiotic resistance, the necessity for novel approaches to combat bacterial infections is felt in numerous fields including agricultural sciences. Numerous important foodstuffs can be infected and destroyed by bacterial pathogens. If pathogens significantly infect essential food crops such as rice, devastating consequences may be imminent for many populations. One agricultural product that fits these criteria is the Nipponbare species wild rice which is infected by *Xanthomonas oryzae* pv. oryzae. This bacterial pathogen uses a transmembrane protein structure called the Type III Secretion System (T3SS) to infect the host plant. The T3SS is a protein structure used by a variety of significant Gram-negative pathogens that serves as a channel to transmit effector proteins into the host cell's cytoplasm en route to invading the cell. For proper function, the protein subunits of the T3SS must be translated and arranged in a precise order. We aim to inhibit the pathogen's ability to infect host cells by obstructing the effective assembly of the T3SS with a nanobody. To do so, we will develop a small protein with the affinity to bind to the Hpa1 protein subunit of the T3SS. Small proteins are notable prospects as food-safe agricultural antibiotics due to their low expense and the human body's ability to metabolize them. In addition, because this antibiotic inhibits infection as opposed to killing the pathogen, it may be possible to target bacteria without contributing to the proliferation of existing antibiotic resistance mechanisms. We herein outline how the processes of yeast cell surface display and directed evolution can be coupled together to identify a protein with the structure and affinity necessary to bind to Hpa1. This biological antibiotic will promote the development of modern antibiotics to confront the growing obstacle of antibiotic resistance.

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Abstract 1278

Progress Toward the Development of Nanobodies related to Moonlighting Activity of *S. aureus* GAPDH via Directed Evolution

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Ryan Beyers, Isabelle Patel, Craig Streu

Antibiotics have transformed healthcare and saved millions of lives. However, with the rapid emergence of resistant bacteria worldwide, decades after the first patients were treated with antibiotics, bacterial infections are once again a threat. A large reason for the surge in resistance is the overuse of common antibiotics. Another reason for this surge in resistance is due in part to moonlighting proteins. These are multifunctional proteins, in which a single protein performs multiple independent functions in different cell compartments, often making use of different conformations to do so. Moonlighting proteins that act as virulence factors tend to play key roles in conserved metabolic processes and elicit relatively muted responses from the immune system. For this reason, moonlighting proteins are hypothesized to be a good target for combating antibiotic resistance. One of the most noteworthy bacteria that exploit these moonlighting proteins is *Staphylococcus aureus*, which contains the moonlighting protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH). This is a key glycolytic enzyme, whose primary function is to aid in the oxidation of glyceraldehyde-3-phosphate (G3P) to 1,3-biphosphoglycerate (1,3-BPG) during glucose metabolism. In extracellular spaces, however, GAPDH has been associated with microbial pathogenicity. One approach to combating this pathogenicity is by interfering with the moonlighting activity of the protein. However, little is known about the origin of GAPDH's moonlighting activity, which highlights the need for chemical probes of extracellular GAPDH function. We herein report our progress toward the development and screening of nanobodies that bind to and modulate the moonlighting activity of *S. aureus* GAPDH via directed evolution.

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Abstract 1281

Role of Hsp90 in regulating sensitivity of *Cryptococcus neoformans* to fluconazole

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Srikripa Chandrasekaran

Cryptococcus neoformans is an encapsulated yeast, found in the lungs of immunocompromised hosts. In the human host, Cryptococcus is subject to multiple forms of environmental stress, including heat stress. Heat shock protein 90 (Hsp90) is an essential protein that has been shown to protect fungal cells against extreme temperature in the human host and to assist in proper cell division. Hsp90 has also been implicated in resistance to the anti-fungal drug fluconazole. To better characterize the role of Hsp90 in fluconazole resistance in Cryptococcus we addressed the following questions: 1. Does concomitant partial inhibition of Hsp90 and treatment with fluconazole lead to increased sensitivity to fluconazole and 2. Does prolonged partial inhibition of Hsp90 prior to treatment with fluconazole lead to subsequent increase in development of fluconazole resistance. Using growth assays, disk assays, and a standardized e-test to determine the Minimum Inhibitory Concentration (MIC) of fluconazole, we found that indeed, pharmacological partial inhibition of Hsp90 with radicicol, concomitant with fluconazole treatment leads to increased sensitivity to fluconazole. However, initial pre-treatment of Cryptococcus with sub-inhibitory concentration of radicicol followed by exposure to fluconazole leads to development of fluconazole resistant Cryptococcus colonies. We characterized the resistant colonies, which show a lower MIC to fluconazole compared to cells that were never exposed to radicicol and fluconazole. Cells derived from the resistant colonies exhibit slower growth on drug-free media, and lose resistance to the drug after passaging in drug-free media. Based on the fact that resistance to fluconazole is lost when fluconazole is removed from the environment, we hypothesize that compromising Hsp90 function during the pre-treatment with radicicol triggers aneuploidy in *Cryptococcus neoformans*.

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Abstract 1300

Characterizing azole drugs in the fungal pathogen *Cryptococcus neoformans*

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Jordyn Wilson, Srikripa Chandrasekaran

Understanding mechanisms of anti-fungal drugs in the pathogenic fungus, *Cryptococcus neoformans*, is crucial for improving anticryptococcal therapy. We have previously determined that presence of anti-oxidants in growth medium can reverse the fungal inhibition caused by the azole drug, fluconazole. However, treatment with the azole drug, fluconazole, presents the problem of resistance of *C. neoformans* to fluconazole treatment. Hence, we screened several azole drugs, including voriconazole, propiconazole, cyproconazole, miconazole, isavuconazole, tebuconazole, myclobutanil, and ketoconazole to determine the following: (1) ability to inhibit growth of H99 strain of *C. neoformans*, (2) MIC of the azole drugs in H99 strain of, (3) ability to induce Reactive Oxygen Species (ROS) in H99 strain of *C. neoformans*, and (4) ability of the anti-oxidant vitamin C to reverse growth of H99 in the presence of each of the drugs. We found that all azole drugs inhibit growth of H99 and we identified the MIC of the drugs against H99. Isavuconazole, voriconazole, and propiconazole showed the largest zone of inhibitions and the smallest MIC among all the drugs tested. However, growth of H99 in presence of these drugs (voriconazole, propiconazole, and isavuconazole) and vitamin C was reversed. Other drugs that showed decreased inhibition of H99 in the presence of vitamin C were ketoconazole and cyproconazole. Inhibitory effects of miconazole, tebuconazole, and myclobutanil were not affected by vitamin C. We found that all the drugs whose inhibitory functions were lowered by vitamin C also induced higher Reactive Oxygen Species (ROS) production in H99. We concluded that while all the eight azole drugs tested inhibited H99 growth, only five drugs (voriconazole, propiconazole, isavuconazole, ketoconazole, and cyproconazole) induced production of ROS in *C. neoformans*.

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Abstract 1303

Avarone and Hymenidin as potential inhibitors for Mycobacterium sp

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Infections of the respiratory tract are increasingly prevalent and drug-resistant. Tuberculosis is one such example. A third of the world's population is infected with tuberculosis, which can become active if the host becomes immune compromised. There is an alarming increase in the number of drug-resistant and extensively drug-resistant strains of tuberculosis. Our study uses the target-specific and whole-cell approach in screening for potential anti-tubercular agents. Our whole-cell approach uses *Mycobacterium smegmatis* as a model (in lieu of the more pathogenic *M. tuberculosis*) and the target-specific approach focuses on the seventh enzyme of the shikimate pathway, shikimate kinase (SK). Substrate-dependent fluorescence binding assays showed a KD of 0.05 ± 0.01 mM. for shikimate and 0.12 ± 0.04 mM for ATP. A KD of 0.26 ± 0.01 mM was recorded for the binding of the inhibitor avarone to shikimate kinase. Chromatograms of the avarone-SK complex showed decreasing intensities over a 24 hr incubation period and the appearance of shoulder peaks, suggesting possible covalent modification of the enzyme. Whole-cell screens with *M. smegmatis* showed an IC50 of 11.89 ± 0.01 and 15.93 ± 0.38 μM for inhibitors avarone and hymenidin, respectively after a 9 hr incubation period. Our data show promising activity for these inhibitors as potential lead compounds and further mechanistic investigation is performed.

I would like to thank and acknowledge East Stroudsburg University for awarding me the Student Undergraduate Research Experience Grant which allowed me to continue to research Avarone and Hymenidin as potential inhibitors for Mycobacterium sp.

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Abstract 1336

Proximate analysis of nutrients and *in vitro* radical scavenging efficacy in selected medicinal plant powders and standard poultry feed

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Lyndy McGaw, Muna Abdalla, Karl Muhling

Evaluation of mineral content and other properties of plant material to generate useful information for promoting plants as antibiotic feed additive alternatives. Concentrations of macro- and micro-minerals in *Morinda lucida*, *Acalypha wilkesiana*, *Ficus exasperata* and standard broiler feed were determined using ICP-MS, and ICP-OES. Degradation of C and N over time were evaluated by dry oxidation (Dumas). Radical scavenging of DPPH and ABTS was investigated and levels of phytochemical constituents were assessed. Macro- and micronutrient concentrations in plants were generally higher than those of standard broiler feed. Total C and N as well as the C/N ratio in plant powders and standard feed were proportional to storage time. Macronutrients were higher in *F. exasperata* and *A. wilkesiana* while micronutrients were higher in *A. wilkesiana* and *M. lucida*. Highest total N and C levels were observed in *M. lucida* while *A. wilkesiana* showed the highest C/N ratio. Remarkable radical scavenging activities were displayed by *A. wilkesiana* while the highest total phenolic content (TPC) and total flavonoid content (TFC) were exhibited by aqueous and acetone extracts of *F. exasperata* respectively. High antioxidant activity was correlated with high total C and C/N ratio of the plant powders. Minerals and organic acids are essential antimicrobial and antioxidant constituents of poultry diets for the maintenance of gut flora health, and for metabolic, enzymatic and antioxidant defence. This study highlights the nutrient content of the selected plants, motivating further investigation of these species as poultry feed additives.

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Abstract 1347

Quorum sensing and developmental mechanisms in African Trypanosomes

Keith Matthews, *University of Edinburgh*

African trypanosomes are protozoan parasites that cause the human disease African 'Sleeping Sickness' and the livestock disease 'nagana' in sub-Saharan Africa, where they are transmitted by tsetse flies. Trypanosomes are extracellular parasites that proliferate in the blood and tissues as morphologically 'slender' forms. These avoid mammalian immune responses by their extreme capacity for antigenic variation, the periodic appearance of new variants and their control by the immune system generating characteristic waves of parasitaemia during the infection. In addition to the immune control of parasite numbers, trypanosomes also exhibit population control through a cell-density dependent, quorum sensing type, phenomenon. Specifically, as numbers increase, the parasites generate a signal that eventually stimulates members of the population to arrest in their cell cycle and develop to morphologically 'stumpy' forms. These arrested stumpy forms are adapted for survival and further life cycle progression once they are taken up in a tsetse fly bloodmeal, such that they assist parasite transmission by, firstly, prolonging the life of the mammalian host through parasite number control and, secondly, by expressing molecules that favour their onward life-cycle development. Over the last few years our laboratory has identified that the signal used by the parasites for cell-to-cell communication comprises oligopeptides generated by parasite-released peptidases. We have also used genome-wide selectional screens to identify molecular components of the signal transduction and gene expression machinery that underlies the quorum sensing response. These have provided molecular insight into the cell signalling mechanisms used by the parasites and have also provided tools to monitor parasite development and inter-species communication of different African trypanosome species in coinfections. In my presentation I will describe the progress made in dissecting the cell-to-cell communication mechanisms used by these parasites using trypanosomes under (i) laboratory selection or (ii) derived from field populations that have naturally lost the ability to undergo quorum sensing and stumpy formation, this promoting their spread outside of the traditional tsetse belt of sub-Saharan Africa. I will also discuss how therapeutic efforts to control the parasite could be threatened by the prevalence of coinfections between different African trypanosome species in natural settings, with the perturbation of the normal interactions between parasite species generating the potential to drive increased disease pathology.

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Abstract 1377

Flagella-mediated antibiotic persistence in *Escherichia coli*

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Michael Gates, Kim Lewis

Persister cells are dormant, antibiotic-tolerant bacterial subpopulations. Unlike resistant cells, persisters cannot grow in the presence of antibiotics and exhibit multidrug tolerance. Because persisters survive antibiotic treatments, they are suggested to be the primary cause of recalcitrant bacterial infections and are more likely to evolve resistance mutations that develop into resistant infections. It is unclear what population heterogeneities encourage individual cells to become persistent or which molecular pathways confer tolerance; however, our lab previously uncovered that flagellar biosynthesis and motility genes are involved in aminoglycoside tolerance. To expand on this research and determine the mechanism by which flagellar genes confer drug tolerance, a complete model of the flagellar gene expression hierarchy was constructed in R via the aggregation of existing CHIP-seq data and curation with the PRECISE genome annotations database. This model was implemented to construct a variety of *Escherichia coli* deletion mutants, with deletions across all levels of the expression hierarchy, that were then subjected to antibiotic killing assays. To verify strain motility phenotypes, a soft-agar motility assay and flagellar rotation assay were adapted from previous literature. It was found that any genetic inhibition of cellular motility, including those impacting highly downstream genes within the flagellar expression hierarchy, both inhibited rotation and motility and significantly reduced persister formation. However, mechanical removal of flagellar filaments via physical shearing had no impact on persisters. Taken together, these results suggest that (1) flagellar gene expression naturally promotes persister cell formation and that (2) genetic disruption of individual flagellar gene expression hierarchy components has broader impacts on either flagellar or global gene expression which suppress antibiotic persistence.

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Abstract 1396

When viral RNA met the cell: a story of protein-RNA interactions

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Wael Kamel, Vincenzo Ruscica, Meghana Madhusudhan, Natasha Palmalux, Shabaz Mohammed

RNA is a central molecule for the RNA virus life cycle as it functions not only as messenger for the synthesis of proteins, but also as storage of genetic information as genome. Given the central role of viral RNA in infection, it is expected that it must function as a hub for critical host-virus interactions. To test this, my laboratory has developed new approaches that have been applied to several viruses such as Sindbis virus, SARS-CoV-2 and human immunodeficiency virus (HIV). We have discovered a new universe of host-virus interactions with central regulatory roles in infection. Interestingly, these viruses, despite having different sequences and infection cycles, engage with a largely shared pool of cellular RNA-binding proteins. My laboratory is currently focused on understanding the regulatory mechanisms underpinning these master regulators with molecular detail. We envision that these central host-virus interactions are promising targets for broad-spectrum antiviral strategies.

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Abstract 1424

Effects of Macromolecular Crowding on the Structure and Activity of Buforin II

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Gabriela Kim, Mala Radhakrishnan, Donald Elmore

Antimicrobial peptides (AMPs) have emerged as a promising alternative to antibiotics in light of the rising problem of antibiotic resistance. The initial step in AMP mechanisms of action involves the peptides interacting with bacterial cell membrane before either disrupting it or translocating into the cell. Lipid vesicles can provide an excellent model system for understanding how AMPs interact with and act on bacterial membranes. Although most vesicle studies are carried out in dilute aqueous buffer conditions, crowders can be introduced to mimic the realistic environment of macromolecules in physiological environments. Similarly, many assays for antimicrobial activity are carried out in dilute buffer conditions. Here, we specifically investigate how antimicrobial peptide secondary structures and activity change in the presence of combinations of lipid vesicles and crowders using a combination of computational and experimental approaches. Previous molecular dynamics (MD) simulations have predicted that the presence of polyethylene glycol (PEG) crowders can lead to a similar enhancement of antimicrobial peptide structural stability as that caused by interactions with a lipid membrane. To follow up on those results, we used circular dichroism spectroscopy to experimentally investigate the nature of AMP structuring in these environments. Preliminary data has experimentally verified the structuring effect on BF2 in the presence of lipid vesicles. Ongoing experimental work in the Elmore lab is adapting our methods for measuring AMPs in the presence of PEG. Other work is utilizing microbroth dilution assays to consider whether crowding also has a direct effect on the antimicrobial activity of BF2. Understanding how AMP structure and activity changes in the presence of crowders provides useful information about how AMPs function in physiological environments.

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Abstract 1439

Not So 'Secret'ome: An Analysis of The Secretion Profiles of *Streptococcus pneumoniae* Secretion Chaperone Mutants

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Laty Cahoon

To be released from the bacteria, proteins must first translocate across the bacterial cell membrane, fold into their final state, and become active in the space between the cell membrane and the cell wall. The membrane-wall space is a challenging environment that inhibits many proteins from folding themselves and necessitates secretion chaperones to perform this function. Investigations into the mechanism underlying bacterial secretion have historically been performed in Gram-negative bacteria which have a thin cell wall sandwiched between inner and outer cell membranes. However, due to structural differences, much remains unclear about the processes responsible for protein secretion in Gram-positive bacteria which have a single cell membrane followed by a thick cell wall. *Streptococcus pneumoniae* is a Gram-positive, extracellular bacterium that is a common inhabitant of the human upper respiratory tract. In *S. pneumoniae*, three secretion chaperones work in the membrane-cell wall space: PrsA, SlrA and HtrA. Preliminary research has determined that each of these chaperones is required for host cell adhesion. This led us to hypothesize that these chaperones are required for the folding and activity of proteins involved in processes such as host cell adhesion. To investigate the varied roles of secretion chaperones, we used proteomic techniques to determine the secretion profiles of ΔprsA, ΔslrA and ΔhtrA mutants. The secretion profiles of these mutants were analyzed to identify proteins that are altered secretion levels in the absence of each secretion chaperone. The "client proteins" identified in these secretion profiles will be investigated for roles in host cell adhesion using biochemical/biophysical and phenotypic approaches.

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Abstract 1442

Shifted Activity of Serine Hydrolases in *Mycobacterium smegmatis* During the Dormant to Active Transition

Allie Goss, *Butler University*

R. Jeremy Johnson

The success of *Mycobacterium tuberculosis* (Mtb), the causative agent of Tuberculosis (TB), can be largely attributed to its ability to enter a dormant, or inactive, state within a host. When infected with dormant Mtb, the host is entirely asymptomatic; however, dormant Mtb can reactivate upon which the host would experience symptoms of TB and become contagious. Most current treatments for TB are ineffective against the dormant growth state, necessitating new therapeutic targets for combating dormant infections and preventing reactivation. One class of enzymes essential to dormant persistence are serine hydrolases, which catalyze the cleavage of lipids—a process essential for both dormancy and reactivation of Mtb. Herein, we show that *Mycobacterium smegmatis* (M. smeg) can be used as a model organism for Mtb by developing a growth system using Nitrogen starvation that mimics the relative changes in serine hydrolase activity and lipid concentrations observed in Mtb upon reactivation. Using TLC for analysis, our growth system showed an increase in TAG concentrations with increasing amounts of supplied carbon (0%, 1%, 2% and 5%), mimicking Mtb's derivation and accumulation of TAG upon infection. It also revealed a decrease in TAG concentrations with increasing activation time (0 hr., 3 hr. or 6 hr.) after being grown in an environment with limited nitrogen, mimicking TAG cleavage upon reactivation. After isolating proteins from M. smeg, exposure to variable fluorogenic ester substrates with a range of chemical compositions was used to identify serine hydrolases exhibiting activity changes across dormant vs active growth conditions. This analysis revealed specific serine hydrolases whose activity changed upon increasing carbon availability and increasing activation time; indicating those hydrolases likely involved in lipid accumulation for persistence and in lipid cleavage for activation. Different serine hydrolase activity could be used to design future therapeutics for Mtb.

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Abstract 1454

A hot mess - radioactive actinides and microbial metabolism

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In this study we assessed the capability of the methylotrophic bacterium *Methylobacterium extorquens* AM1 to use lanthanides and radioactive actinides for growth on ethanol. Lanthanide metals were recently added to the biological periodic system of elements that support life. Since then, lanthanides, part of the f-elements along with actinides, have been shown to function as Lewis acid cofactors in certain PQQ-alcohol dehydrogenases found in methylotrophic bacteria. We chose to use a genetic variant of *M. extorquens* AM1, evo-HLn, that can only produce the f-element-dependent ExaF dehydrogenase for ethanol oxidation. Because this strain has evolved the capability to grow with the heavy lanthanide gadolinium, we hypothesized it could use an expanded range of heavier actinides for growth. Phenotypic growth analyses show that actinides of similar ionic radii and oxidation state (actinium, americium, curium) can support growth at similar rates to the light lanthanides, and heavier actinides with smaller ionic radii (berkelium and californium) support growth at rates similar to the heavy lanthanides europium and gadolinium. This indicates the ionic radius of the metal directly influences growth, most likely through modulation of ExaF ethanol dehydrogenase activity. ExaF was purified without the metal component of the cofactor complex and activity was reconstituted *in vitro* with a lanthanide or actinide and PQQ. Enzyme activities were measured using an *in vitro* dye-linked assay, exhibiting decreased activity across the lanthanide series. In contrast, ethanol dehydrogenase activity increases across the actinide series, though overall actinide activities are lower than lanthanide activities. This indicates that Lewis acidity also likely plays a role, along with the ionic radius, in how effective the metal is in the active site. Metal uptake, measured by ICP-MS in cell-free extracts, during ethanol growth increases across the lanthanide series, confirming that evo-HLn has evolved an increased capacity for uptake of heavier metals. Increased heavy metal uptake, specifically of heavy lanthanides and radioactive actinides, provides a novel mechanism for the potential bioremediation of contaminated sites and materials.

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Abstract 1463

Outcomes Associated with Bacterial Species Detected in Blood Exomes of Melanoma and Cervical Cancer Patients

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Bacteremia poses great risk for morbidity and mortality for immunocompromised cancer patients. Though the presence of bacteria within solid tumors is gaining greater attention, very few studies have analyzed species of bacteria in the blood and their effect on cancer clinical outcomes. Using the Kraken 2 taxonomic profiling tool and bacterial genomic libraries downloaded from the National Center for Biotechnology Information (NCBI) Reference Sequence database, we classified bacteria present in blood and primary tumors of the cervical cancer and melanoma cases. The Cancer Genome Atlas (TCGA) melanoma blood exome files with Pseudomonas species were found to represent a worse disease-free survival (DFS) probability, while a worse overall survival (OS) result was evidenced for both the TCGA and Moffitt Cancer Center melanoma datasets. Cervical cancer cases with reads representing the Bradyrhizobium genus and Bradyrhizobium sp. BTAi1 found in blood and tumor exomes were found to have lower DFS. Meanwhile, reduced DFS and OS was found for cases represented by tumor exomes of cervical cancer cases positive for Bacteroides species including *Bacteroides fragilis*. This study provides novel evidence and a novel approach for determining that bacteria in blood is associated with cancer recurrence. Our findings may guide the development of prognostic and screening tools related to bacterial blood infections for melanoma and cervical cancer patients.

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Abstract 1469

Optimizing the Isolation of Bacterial Outer Membrane Vesicles from Complex Biofluids

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Sepsis is a clinical syndrome that occurs when host proinflammatory immune responses become elevated in response to an infection. If untreated, sepsis can result in severe sepsis, causing organ failure or hypotension, known as septic shock. Outer membrane biomolecules LPS, OmpA, and Pal have been implicated in the pathophysiology of *Escherichia coli* (*E. coli*) sepsis, specifically, the over-inflammatory response observed in sepsis patients. Notably, all of these biomolecules are released as a complex inside outer membrane vesicles (OMVs) from *E. coli* and other Gram-negative bacteria. We hypothesize that these biomolecules and OMVs themselves would serve as excellent biomarkers for the diagnosis of sepsis. Since we aim to isolate OMVs from patient biofluids, such as human plasma, we set out to optimize the isolation protocol to recapture previously purified *E. coli* OMVs spiked into healthy human plasma. We detected and characterized the isolated OMVs using immunoblotting, nanoparticle tracking analysis, and electron microscopy. Although the exact levels of OMVs in sepsis patient biofluid are currently unknown, the results from our current optimized OMV isolation method suggests that we can capture OMVs from a complex biofluid such as human plasma without significant false negatives.

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Abstract 1471

The effect of antibiotics on the production of outer membrane vesicles and apoptotic bodies

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Extracellular vesicles (EVs) are nanoscale, lipid-bound species released from prokaryotic and eukaryotic cells. Gram-negative bacteria release several different types of EVs, including outer membrane vesicles (OMVs) and apoptotic bodies (ApoBDs). OMVs are EVs derived from the outer membrane of Gram-negative bacteria and often contain biomolecules, such as proteins, nucleic acids, and lipids, from their parent cell. ApoBDs, which also contain molecules from their parent cell, are EVs released by cells that are in the process of regulated programmed cell-death or apoptosis. Bacterial ApoBDs are a physiological marker of cell death-inducing stressors, such as antibiotics. OMV production is known to increase in the presence of environmental stressors. Although these two entities are structurally and mechanistically distinct from each other, common isolation methods likely result in co-purification of OMVs and ApoBDs. Here, we describe our study, which seeks to elucidate the effect of different types of antibiotics on the production of OMVs and ApoBDs in *Escherichia coli*. We employ ultracentrifugation to isolate these EVs and immunoblotting, electron microscopy, and nanoparticle tracking analysis to differentiate between the two EV types. Results from preliminary experiments show that both OMVs and ApoBDs are increased in the presence of antibiotics, and that differentiation between the two EV types remains a challenge. With the emergence of novel biomedical applications for OMVs, better isolation and identification protocols are needed to differentiate between OMVs and ApoBDs.

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Abstract 1500

An engineered bacteria expressing an n3-fatty acid desaturase altered polyunsaturated fatty acid levels in mice

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Background: The Western diet is heavily represented by foods rich in n6 polyunsaturated fatty acids (n6 PUFAs, e.g., from corn and soybeans) and with fewer sources of n3 PUFAs (e.g. certain plants, nuts, and oily fish). This PUFA imbalance is relevant to overall health because n6 PUFAs such as linoleic acid are metabolized to pro-inflammatory lipids, whereas n3-PUFAs produce anti-inflammatory lipids. Diets with a high n6:n3 PUFA ratio contribute to the development of chronic inflammatory diseases and cardiovascular disease. Dietary supplements are a popular approach to increase n3 PUFA consumption (e.g., fish oil capsules); however, compliance is often low, and lack of quality control and potential heavy metal contamination are concerns. Herein, we engineered *Limosilactobacillus reuteri* 6475 (LR) to express an n3-fatty acid desaturase enzyme (FAT1), which catalyzes the conversion of n6 PUFAs to n3 PUFAs.

Methods: The *C. elegans* n3 fatty acid desaturase (fat1) gene, was cloned into a multi-copy plasmid and stably-expressed in LR to yield LR-Fat1. LR harboring an empty plasmid was used as a control (LR-Con). The intracellular accumulated FAT1 is released from the engineered bacteria following activation of prophages, which leads to cell lysis. Importantly, the prophages are naturally activated during gastrointestinal transit. Expression of the fat1 gene and production of the FAT1 protein were determined by qPCR and SDS-PAGE, respectively and growth curves performed. Germ-free (GF) and conventional C57BL/6J mice were gavaged with either PBS or 109 LR-Con or LR-Fat1. Plasma and tissue levels of fatty acids were measured by mass spectrometry and fecal samples were analyzed for LR and associated prophages.

Results: SDS-PAGE and qPCR demonstrated that FAT1 was expressed in LR-Fat1, although growth curves demonstrated a small growth disadvantage of LR-Fat1 vs. LR-Con. GF mice, which can stably colonize LR and require only a single gavage, had only minor changes in fatty acid profiles, most notably an increase in hepatic levels of n3 PUFAs, compared to LR-Con. Conventional mice do not stably colonize LR and require multiple gavages. Therefore, conventional C57BL/6J mice were gavaged each day for 10 days with LR-Fat1, and revealed greater changes in fatty acid levels, including increased plasma levels of multiple n3 PUFAs, including EPA, DPA, DHA, and HDHA. We also identified increased plasma levels of n3 PUFA metabolites including 8,9-EpETE, 11,12-EpETE, 14,15-EpETE, 17,18-EpETE, 5-HEPE, 15-HEPE, and 18-HEPE. There also was an increase in n3 PUFA levels in the liver and cecum contents. Analysis of fecal samples from both GF and conventional mice confirmed the presence of both bacteria and phage.

Conclusions: These results demonstrate that mice treated with LR-Fat1 had a shift in their fatty acid profiles to n3-PUFAs and corresponding, potentially anti-inflammatory metabolites. This novel engineered probiotic LR-Fat1 bacteria has potential implications in the treatment of diseases with underlying chronic inflammation.

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Abstract 1515

Co-Culture of Bacteria with Primary Human Colonic Epithelium in a Mucus-Covered 2D Crypt Model

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Introduction: A complex and dynamic network of interactions exists between mucus in the human gastrointestinal tract and the intestinal microbiota. An inner mucus layer acts as a physical barrier to separate intestinal bacteria from host tissue, while the outer mucus layer is permeable to microbes and can even be utilized as a nutrient source by some microbes. In contrast, intestinal pathogens can damage the integrity of these mucus layers. Other bacteria, such as probiotics, may counteract pathogenic bacteria by boosting mucus production. Therefore, understanding microbe-mucus-epithelial cell interactions is critical for the understanding and treatment of intestinal diseases.

Method: We established a 2-dimensional (2D), self-renewing human intestinal microphysiological system. In this 2D crypt model, primary human colon stem cells were seeded onto an array of microholes formed between luminal and basal fluid reservoirs. When growth factors such as Wnt, R-spondin and Noggin were placed into the basal reservoir, only the cells directly overlying the microholes received these growth factors. Under these conditions, a stem cell niche formed above the microholes while cells elsewhere differentiated, forming colonocytes, goblet cells and other lineages. A 100 μm thick mucus layer spontaneously formed across the surface of these crypts when the array was held in culture for 10–15 days. Microbes such as *Staphylococcus aureus*, *Lactobacillus rhamnosus GG*, and *Clostridium scindens* grew in the luminal compartment under either aerobic or anaerobic condition (with an oxygen gradient cassette). Epithelial cell viability, monolayer integrity, and mucus thickness were monitored in response to the presence of microbes. The ability of the mucus layer to prevent microbe invasion into the epithelial layer was assessed.

Results: The presence of mucus significantly improved the viability of colonic epithelial cells co-cultured with *S. aureus* at 24 h. The mucus also reduced damage by *Clostridioides difficile* toxin TcdA to colonic cells, although the integrity of the epithelial monolayer and cell cytoskeleton were partially disrupted (e.g., ZO-1 and F-actin proteins) after prolonged exposure to the toxin. The intestinal probiotic strains *L. rhamnosus GG* and *C. scindens* grew on the outer layer of mucus with no noticeable negative effects on colonic cells, thus establishing a microbial barrier that might serve to hinder pathogen invasion.

Conclusion: In this study, interactions between human intestinal mucus and key members of the microbiota were replicated in the mucus-covered 2D crypt model. This 2D platform provides an innovative *in vitro* microphysiological system for studying the interactions of microbes with mimic of a human large intestine. Importantly the impact of microbes, microbial products (toxins and metabolites), pharmaceutical compounds, and food products can be assessed for their interactions with the mucus layer, colonic stem and proliferative cells, and the differentiated cell types.

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Abstract 1555

Isolation of *M. smegmatis* bacteriophage lysin B and characterization of its potential as an antimicrobial agent for *M. tuberculosis*

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Michael Wolyniak

Phages are viruses that infect bacterial cells and depend on them for replication. The recent surge in antibacterial-resistant bacteria has brought attention to bacteriophages as an alternative treatment for pathogenic bacterial infectious diseases such as gonorrhea, pneumonia, tuberculosis, leprosy, and wound infections. This technique, called phage therapy, has been available since to treat dysentery in 1919 by Felix D'Herelle. Recently, scientists have demonstrated that bacteriophages can be used to successfully reduce Salmonella species in meat and poultry products, a discovery that reignited interest in phage therapy as a viable means of controlling pathogenic bacterial populations. To further explore the potential for phage therapy as a means of fighting pathogenic bacterial infections, we isolated phage LestyG from *Mycobacterium smegmatis*, a close relative of *Mycobacterium tuberculosis*, the bacterium responsible for tuberculosis. After isolating and sequencing the LestyG genome, bioinformatic characterization was performed using the PECAAN (Phage Evidence Collection and Annotation Network) platform. Further analyses were made on the annotated genes by comparing the genome of LestyG to that of DS6A—the only bacteriophage discovered from *Mycobacterium tuberculosis*. It was discovered that LestyG has one gene in close homology with DS6A (only phage discovered from *M. tuberculosis*): gene 48 in LestyG and gene 72 in DS6A. Gene 48 codes for lysin B, a mycolylarabinogalactan esterase that cleaves the ester bond between arabinogalactan and mycolic acid and, thus, compromises the link between the mycobacteria cell wall and the outer membrane to completes cell lysis. Lysin B has the ability to hydrolyze unbound mycolic acids in different mycobacteria species, including *Mycobacterium tuberculosis*. Because of the potential in lysin B for disrupting the cell wall structure of pathogenic bacteria, it became the focus of our research. We have successfully cloned the LestyG Lysin B gene into a protein expression vector for the purposes of characterizing the ability of this protein to influence Mycobacterium pathogenesis. Current research focuses on optimizing the conditions for Lysin B protein exposure to Mycobacterium with the goal of disrupting replication and reducing the bacterial population. We intend to extend this investigation into the effects of Lysin B on an attenuated strain of *M. tuberculosis* to determine whether lysin B compromises the integrity of the cell wall of *M. tuberculosis*, thereby weakening its resistance to antibiotics. This experiment is ultimately aimed to be the foundational model of the use of lysin B in conjunction to antibiotics to effectively treat tuberculosis and other pathogenic bacteria.

Hampden-Sydney College Office of Undergraduate Research.

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Abstract 1569

Assessing the Cholinergic Anti-Inflammatory Response in Human Macrophages Exposed to the Spike Protein of the SARS-CoV-2 Strain from Wuhan, China

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Manuel Delgado-Vélez, Negin Martin, Jerrel Yakel, José Lasalde-Dominicci

To date, the severe acute respiratory syndrome coronavirus 2 that causes the disease Coronavirus 2019, has infected 601 million people, claiming the lives of 6.4 million people worldwide. Of the patients who survive, 60% suffer from inflammatory problems leading to post-acute sequelae of COVID-19 (PASC). Inflammation in these patients is marked by an increase in pro-inflammatory cytokines which ultimately damage the body's organs, contributing to PASC. Understanding the main mechanism by which this cytokine storm occurs is of utmost importance in order to develop therapeutic strategies for counteracting inflammation in people suffering from COVID-19 and PASC. This project seeks to find out if an innate anti-inflammatory mechanism, the cholinergic anti-inflammatory response (CAR), works properly in patients suffering from COVID-19 and PASC by interrogating its functioning in its cellular substrate, macrophages. We hypothesized that disruption of the CAR in primary human monocyte-derived macrophages (MDMs) exposed to the SARS-CoV-2 spike protein trimer contributes to the chronic inflammation/cytokine storm exhibited in these patients. To this end, we exposed MDMs to the SARS-CoV-2 spike protein in order to assess levels of the anti-inflammatory alpha-7 nicotinic acetylcholine receptor (α7-nAChR) by means of confocal imaging. Our results demonstrate a statistically significant reduction ($P \leq 0.01$) of α7-nAChR expression in MDMs, in a time-dependent manner, after the addition of SARS-CoV-2 spike protein concentrations (30 nM and 100 nM), at different time points. Interestingly, when the receptor employed by the virus to infect, Angiotensin-converting enzyme 2 (ACE-2), was blocked, we detected a significant reduction in the levels of α7-nAChRs ($P \leq 0.001$). Collectively, our results support the hypothesis of this work given that the SARS-CoV-2 spike protein is capable of compromising the functioning of the CAR by reducing the levels of α7-nAChRs available in macrophages to suppress inflammation. These results could position the α7-nAChR as a key target for the development of novel anti-inflammatory therapeutic strategies to counteract the inflammatory problem found in patients suffering from COVID-19 and PASC.

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Abstract 1580

Understanding the Mechanism of Phage-Mycobacteria Interaction

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Lia Danelishvili

Mycobacterium abscessus belongs to the group of a nontuberculous mycobacteria and is the significant pathogen causing pulmonary infections in Cystic Fibrosis (CF) patients. *M. abscessus* has natural resistance to most available antibiotics, limiting the therapy options in clinics. Bacteriophages (phages) are promising alternatives to antibiotics that can effectively kill drug resistant pathogens. In few cases, the phage therapy has been used to treat disseminated and chronic bacterial infections in CF patients. Phage binds/adsorbs to diverse cell surface molecules as receptors on bacteria, infect the host by injecting the DNA and, later, bursting cells through lysis. Due to the complex cell wall structure of mycobacteria, the research on phage adsorption mechanisms have been limited. Recent literature on Gram-negative bacteria describes that phages can utilize porin proteins as receptors. Interestingly, porin proteins are present on the cell wall surface of mycobacteria and are associated with antibiotic import in bacterial cell. Our hypothesis is that antibiotic combination with phages that utilize porins as receptors potentially can diminish the drug efficacy (instead of improving). To test our hypothesis, at first, we aimed to identify phages that bind to porins as receptors. A library of mycobacterial phages was screened against *M. abscessus* in presence of two porin inhibitors (spermidine and cadaverine) and phages that no longer could adsorb on bacteria (in comparison to no porin inhibitor control group) were recognized as phages with binding mechanism to porins. Our screen identified 4 phages out of 132 that lost ability to effectively invade *M. abscessus* after treatment with porin inhibitors. Next, to validate our hypothesis, we used these selected phages and combined with amikacin treatment (a frontline drug for *M. abscessus*). Preliminary results suggest that while phage and antibiotic treatment alone significantly reduce *M. abscessus* growth *in vitro*, the phage-antibiotic treatment combination delays bacterial killing. This study suggests that some lytic phages may have antagonist effect when combined with antibiotics and, therefore, the understanding the phage-bacteria interaction mechanisms is essential in developing the most effective phage therapies.

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Abstract 1586

The role of lymphocyte cytosolic protein 1 in a *Mycobacterium marinum* infection in *Danio rerio*

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Multidrug-resistant tuberculosis remains a growing and persistent threat to global health. The World Health Organization reported 310 000 cases of multidrug-resistant tuberculosis in 2011 and an increase to 450 000 cases in 2021, reflecting a need for new therapeutic avenues such as host-based therapies. Toward this end, we sought to investigate the role of the host gene lcp1 in tuberculosis pathogenesis. lcp1 encodes the protein product, lymphocyte cytosolic protein 1 (LCP1 or l-plastin). LCP1 is expressed exclusively in hemopoietic cells, including macrophages, which are the targets of *Mycobacterium tuberculosis*. LCP1 is an actin-binding protein theorized to influence macrophage motility, however, the role of LCP1 in tuberculosis pathology is not known. Using the zebrafish-*Mycobacterium marinum* model of tuberculosis we sought to gain a better understanding of the effect of LCP1 mutation on bacterial burden. We obtained an LCP1 mutant zebrafish line and developed an assay capable of genotyping hundreds of larvae per experiment allowing us to observe the difference in bacterial burden with appropriate precision. We performed an infection of 2-day post-fertilized larvae from an LCP1 +/− and −/− incross with tdTomato expressing *Mycobacterium marinum* and implemented our assay 5 days post-fertilization. Through the quantification of bacterial fluorescence, we observed a general trend toward greater bacteria burden in LCP1 −/− larvae. Understanding why fish without LCP1 displayed a higher bacterial burden could provide insight into the role LCP1 plays in the human body's immune response to tuberculosis. The future directions of this project include observing more fish in order to replicate the findings and to test other phenotypes such as granuloma size, macrophage migration towards the infection site, and macrophage longevity.

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Abstract 1639

Rapid PCR-based Genetic Typing System across *Chlamydia trachomatis*

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Chlamydia trachomatis causes serious disease of the eye and genital tract in millions of people. Despite differences in tropism and presentation, there is a remarkable level of sequence conservation within the species. Using existing genomic sequence databases, we have explored genomic and protein conservation within *C. trachomatis* and across the Chlamydia genus. We collected and organized read data sets from online nucleotide archives; bioinformatically mapped database reads to a common reference strain genome assembly; computationally characterized each conserved nucleotide sequence; and generated a database of predicted peptides unique to and completely conserved within the species which allowed us to globally map conserved sequences. We used fully conserved nucleotide sequences identified in our analysis to design species-specific primers for genome sequencing of clinical *C. trachomatis* strains. Examination of the global *C. trachomatis* nucleotide sequence database identified over 40 000 completely conserved nucleic acid sequences (max length = 467 bp). The conserved nucleic acid sequences were translated in 6 frames, which provided a database of predicted peptide conservation across the species. Species-specific primers designed using the conserved nucleotide sequences encompassing 10% of the *C. trachomatis* genome (~100kbp) successfully amplified the sequences from an uncultured clinical sample. We then used a PCR-based method to analyze non-cultured *C. trachomatis* in nonviable remnant diagnostic specimens. Contemporary diagnostics for Chlamydia infection do not currently include culture of clinical *C. trachomatis*, but bioinformatic characterization of *C. trachomatis* within specimens allow detailed analysis of specific strains found in an infected patient. This analysis has led to a database of useful peptide and nucleotide sequences with full conservation that will be useful for both basic and applied investigations in chlamydial biology.

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Abstract 1644

Enhanced OMV release from *E. coli* in the presence of beta-lactam antibiotics

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Nico Burgado, James Crawford, Gabriela Gonzalez, Panteha Torabian, Tom Gaborski, Lea Michel

Outer membrane vesicles (OMVs) are nano-sized, membrane-bound spheres constitutively released by Gram-negative bacteria. OMVs contain many similar molecules to their cell "parent," some of which are toxic and have been shown to cause inflammation in host tissue. Studies have shown that OMV production is enhanced by the presence of environmental stressors like antibiotic exposure. We hypothesized that some antibiotics, depending on their mechanism of action, might enhance OMV release from Gram-negative *Escherichia coli* (*E. coli*) more than other antibiotics. After incubation of *E. coli* with each of nine antibiotics at twice their minimum inhibitory concentrations, we isolated the OMVs using ultracentrifugation and quantified them using immunoblotting, nanoparticle tracking analysis, and electron microscopy. Our results suggest that some antibiotics, specifically beta-lactams, do indeed enhance OMV release more than others do. Since OMVs themselves, as well as many of molecules inside OMVs, can trigger inflammation, these results may have implications for doctors who prescribe antibiotics to sepsis patients.

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Abstract 1647

Quantification of functional antibody titers in a mouse model of SARS-CoV-2 infection

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Rakhi Harne, Brittany Williams, Susan Baldwin, Rhea Coler

Towards the end of 2019 a novel severe acute respiratory syndrome (SARS)-like coronavirus (SARS-CoV-2) caused the ongoing global pandemic. The virus surface consists of spike proteins that mediate SARS-CoV-2 entry into cells through its receptor-binding domain (RBD) that attaches to the human receptor Angiotensin- Converting Enzyme 2 (ACE2). Upon infection with foreign material, like viruses and bacteria, the human immune system responds by producing a humoral response specific to the viral antigen. Cells from the innate immune system and antibodies generated in the humoral response work to destroy and block infectious antigens from causing damage to the human cells. The S protein of SARS-CoV-2 is the key protein that stimulates the immune system to generate neutralizing antibodies. To safely test and investigate SARS-CoV-2 in BSL-2 lab setting, we propagated a surrogate pseudo typed virus to evaluate the ability of antibodies to reduce viral cell entry and replication in SARS-CoV-2 infected mice model. Quantifying the functional ability of neutralizing antibodies would help us understand how they influence reinfection in recovered individuals. We hypothesize that antibodies generated in SARS-CoV-2 infected mice models will induce a protective immune response against the SARS-CoV-2 infection. To detect and quantify the protective immune response generated in mice, we performed two different serological assays and identified antibodies endpoint titers. Mice were infected with Delta and Beta at time points Day 3 and Day 4. We performed a SARS-CoV-2 Spike pseudo virus neutralization assay and measured luminescence to determine the percentage neutralization of functional antibodies induced in mice serum samples upon infection. Utilizing indirect ELISAs,' we measured absorbance for IgA antibodies in Bronchoalveolar lavage fluid (BALF) serum and total IgG antibodies in cardiac bleeds. Our results showed we did not obtain neutralizing activity of antibodies in mice serum samples taken at early time points, 24 hrs and 4 days, after infection with the Delta variant of SARS CoV2 virus using both the pseudo viruses Omicron and WA spike. We obtained 100% neutralizing activity in mice serum samples taken at day 21 and infected with Beta variant of SARS CoV2 virus using both the pseudo viruses Omicron and WA spike demonstrating that there is cross-neutralization against various variants of concern. Antibodies (IgA, IgM, IgG) generated in mice 3 weeks post infection with SARS CoV2 (Beta) virus are capable of neutralizing and inhibiting the entry of WA spike and Omicron pseudo viruses in human HEK293 T Ace2 cells. Moving forward utilizing samples with timepoints surpassing 3 weeks could possibly

yield higher concentrations of IgA and IgM antibodies that can neutralize the SARS-CoV-2 pseudo virus.

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Abstract 1657

Design and Evaluation of Buforin II Variants with Enhanced DNA Binding and Antimicrobial Activity

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Qiao Li, Lisha Jing, Mala Radhakrishnan, Donald Elmore

Antibiotic resistance (AR) poses one of the greatest threats to global health, food security, and economic development. Typically, when treating bacterial infection, physicians can prescribe patients with antibiotics–until the bacteria develop AR, and the antibiotic stops working. Recent research has demonstrated that antimicrobial peptides (AMPs) may serve as possible alternative treatments for infection. AMPs are convenient and promising because they are already an important part of the immune system in various living organisms, including humans, and they often have a different mechanism of action to kill bacteria than conventional antibiotics. One AMP, buforin II (BF2), works by entering the bacterial cell membrane and binding to the DNA and RNA of the cells. To optimize DNA-binding and consequently antibacterial properties of BF2, we used a combination of molecular dynamics simulations and electrostatics calculations to design mutant peptides that have greater electrostatic interactions with DNA. These computations predicted enhanced DNA binding for T1R and L8R substitutions, with T1R having stronger predicted binding. We confirmed our computational predictions by experimentally measuring DNA-binding strength using fluorescence intercalator displacement (FID) assays. These experiments confirmed that while both mutations enhanced DNA binding, the effect was stronger for T1R. Lastly, we tested to see if the peptides also followed the same trend in activity via radial diffusion and microbroth dilution assays with *E. coli*. Our findings indicate that the T1R and L8R BF2 mutants have either similar or significantly improved activity compared to the WT BF2 peptide, suggesting that it is possible to design AMPs to be more effective against bacteria. These results also confirm the Future directions include incorporating macromolecular crowders into media to more accurately depict physiological conditions and using advanced microscopy to determine whether the mechanisms of action for the mutant peptides remain the same or differ from the WT BF2 peptide.

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Abstract 1659

Enhanced vesiculation from *E. coli* in the presence of beta-lactam antibiotics

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Navraj Singh, Nico Burgado, Gabriela Gonzalez, Panteha Torabian, Tom Gaborski, Lea Michel

Sepsis is a leading cause of death in hospitalized patients. During sepsis, an initial infection, often bacterial, induces an overactive immune response that can culminate in organ failure or septic shock. Although there is no cure, one common treatment for sepsis is broad range antibiotics to eliminate the bacterial infection source. However, one negative side-effect of antibiotic treatment may be the enhanced released of outer membrane vesicles (OMVs) from Gram-negative bacteria such as *Escherichia coli* (*E. coli*). OMVs are membrane-bound nanoparticles that allow for the transport of proteins, nucleic acids, and other biological molecules from one bacterium to another. OMVs also notably contain toxins, some of which have been shown to be associated with sepsis-related inflammation. Here, we hypothesize that certain classes of antibiotics may result in an increased release of OMVs depending on their mechanism of action. In our experiments, we incubated a clinical strain of *E. coli* with each of 9 antibiotics from various classes, including beta-lactams, at twice their respective minimum inhibitory concentrations, and quantified the released OMVs by immunoblotting. The results of this study suggest that certain antibiotics do enhance OMV release from *E. coli* more than other antibiotics. These results may be an important consideration when considering which type of antibiotic to administer to sepsis patients.

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Abstract 1673

Inhibiting the Intramembrane Aspartic Acid Protease PilD: Targeting a lynchpin of virulence in *Pseudomonas aeruginosa*

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The World Health Organization's prognoses that the world is on the precipice of a "post-antibiotic" era underscores the critical need for antibiotic drug innovation; this includes novel drug targets in pathogens of concern and a reconceptualization of approaches to treating infections from anti-biotic to anti-infective strategies. One new target is the GxGD-type intramembrane cleaving aspartic acid protease PilD, which is essential for the assembly and function of both the Type 4 Pilus and Type 2 Secretion System in the opportunistic human pathogen *Pseudomonas aeruginosa*. PilD, however, is not essential for survival. While this enzyme has been biochemically characterized, PilD has never been systematically investigated as a drug target despite the apparent advantages of affecting multiple virulence phenotypes simultaneously. We are developing a high-throughput FRET-based PilD peptidase assay to screen potential inhibitors. Initial screening will be performed with an FDA-approved drug library, selected inhibitors of other aspartic acid proteases, and rationally designed peptides and peptidomimetics derived from the native PilD substrate. An orthogonal post-peptidase PilD methyltransferase inhibition assay is being optimized to validate initial inhibitor candidates. Work is ongoing to optimize the FRET-assay for high-throughput application and improve the orthogonal assay robustness. Inhibitors will be validated as anti-infective leads through bacteriostatic and bactericidal counter screens, and *in vivo* efficacy will be confirmed in a *C. elegans* infection model. This inhibitor screening and validation will identify the first specific inhibitors of PilD, provide insights into inhibiting intramembrane cleaving aspartic acid protease, and may produce new anti-infective drug candidates for the treatment of *P. aeruginosa* infections.

This project was supported in part by a fellowship award through the National Defense Science and Engineering Graduate (NDSEG) Fellowship Program, sponsored by the Air Force Research Laboratory (AFRL), the Office of Naval Research (ONR) and the Army Research Office (ARO).

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Abstract 1689

Utilizing biomarkers to differentiate *E. coli* apoptotic bodies (ABs) from outer membrane vesicles (OMVs)

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Outer membrane vesicles (OMVs) are 20–250 nm particles released from Gram-negative commensal and pathogenic bacteria through outer membrane budding. OMVs are thought to promote bacterial communication and pathogenicity. In contrast, apoptotic bodies (ABs) are vesicles produced during programmed cell death, a bacterial SOS response that involves activation of the RecA-LexA mediated pathway. Although ABs can be quite large (hundreds of nm in diameter), there can also be ABs that are similar in size to OMVs, making it difficult to separate and differentiate between the two populations. Our lab's research focuses on the role of bacterial OMVs in sepsis, but we acknowledge that differentiating OMVs from apoptotic bodies may be a challenge. In addition, there are similar environmental conditions that enhance both OMV and AB production, such as the addition of antibiotics to bacterial cultures. Considering these challenges, we hypothesize that we can utilize AB biomarkers, such as RecA, LexA, and Annexin V, to differentiate ABs from OMVs. Our methods for visualizing these biomarkers include immunoblotting, nanoparticle-tracking analysis, and electron microscopy. Preliminary results show increased expression of RecA in *Escherichia coli* (*E. coli*) cells cultured in the presence of apoptotic agent and antibiotic, ampicillin, as well as increased expression of OMV biomarkers such as lipopolysaccharide (LPS), outer membrane protein A (OmpA), toll-like receptor B (TolB), and peptidoglycan-associated lipoprotein (Pal). These results suggest that the production of both OMVs and ABs are increased by ampicillin, but that we may be able to differentiate between the two populations.

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Abstract 1703

Effects of Walnut Consumption on Gut Microbiota Function and Composition

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Background: The effects of walnut consumption on the gut functionality that leads to reduction of CVD risk factors has not been previously studied. Objective: The aim of this study is to examine the differences in the gut microbiota composition and functionality between-diet groups in individuals at increased cardiovascular risk and to determine the implications of walnut consumption on cardiovascular health.

Design: Forty-two individuals at cardiovascular risk were put on a randomized controlled feeding trial that included a 2-wk Standard Western diet (run-in) (50% kcal carbohydrate, 16% protein, 34% fat, 12% SFA) followed by three 6-wk isocaloric study diets: walnut diet (WD; 57–99 g/d walnuts; 2.7% ALA); walnut fatty acid-matched diet without walnuts (WFMD; 2.6% ALA); and oleic acid replaces ALA diet without walnuts (ORAD; 0.4% ALA). Fecal samples were collected from all the individuals which were then subjected to metatranscriptomic analysis to investigate the gut microbiota composition and functionality.

Results: Alpha diversity assessed by the Observed ASVs (SAS; $p = 0.27$) or Pielou's Evenness (SAS; $p = 0.09$) showed a significant impact of diet when active composition was considered. Compared to the run-in, alpha-diversity of the active composition did not differ following the diets (SAS; $p > 0.05$). There were no between-diet differences in beta-diversity for the active composition (Adonis; $p = 0.59$) or the genes (Adonis; $p = 0.92$). LEfSe (Linear Discriminant Analysis Effect Size) between-diet comparisons showed enrichment of several genera and species from the Actinobacteria and Firmicutes phyla following the WD, WFMD and ORAD relative to run-in. Our findings also suggest the enrichment of Gordonibacter (Wilcoxon Signed Rank; $p < 0.001$) following the WD, WFMD and ORAD relative to the run-in. This bacterium is responsible for converting ellagitannins and ellagic acid to urolithins which is the primary form in which ellagitannins are absorbed by our body. MaAsLin2 (Microbiome Multivariable Association with Linear Models) analysis showed the enrichment of several important genes that mapped to important metabolic and biosynthetic pathways in the WD group compared to run-in.

Conclusions: Walnut consumption led to shifts in gut microbial composition and induced the expression of beneficial genes and pathways that have further health implications and provides possible explanations for reduction of CVD risk factors.

This study was funded by The California Walnut Commission. This research was also supported by the Penn State Clinical and Translational Research Institute, Pennsylvania State University Clinical and Translational Science Award and NIH/National Center for Advancing Translational Sciences (grant no. UL1TR000127).

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Abstract 1733

Metformin Degrading Bacteria: Genomes, Metabolic Products, and Transcriptional Regulation

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Metformin is one of the most prescribed pharmaceuticals worldwide, mainly for treating type 2 diabetes. This pharmaceutical is a major anthropogenic pollutant that accumulates in waterways at extremely high rates, negatively affecting aquatic life. A novel metformin-biodegrading bacteria known as *Pseudomonas mendocina* strain MET was recently isolated from a wastewater treatment plant. The genome of this bacterium encodes two novel enzymes, GbuAB and GuuH, essential for metformin degradation. The goal of this study was to conduct a complete genomic and physiological characterization of *Pseudomonas mendocina* strain MET and investigate the transcriptional regulation of the enzymes involved in metformin degradation. We hypothesize that strain MET can grow in and metabolize compounds structurally similar to metformin, and that this drug induces the expression of genes encoding metformin degrading enzymes. Metformin and structurally related compounds were used in growth studies to evaluate the metabolic capabilities of *P. mendocina* strain MET. Whole genome sequencing and bioinformatics analyses were performed to examine the DNA regions encoding the metformin-degrading enzymes and to carry out comparative genomics among closely related bacteria. Total RNA extractions and quantitative RT-PCR studies were done to investigate the transcriptional regulation of the GbuAB and GuuH enzymes. The results demonstrated that *Pseudomonas mendocina* strain MET had the ability to completely metabolize metformin and other biguanide compounds, including 1-N-methylbiguanide, biguanide, guanylurea, and guanidine, into equivalents of carbon dioxide and ammonia. Quantitative RT-PCR analysis revealed that the genes encoding the metformin-degrading enzymes were expressed at approximately the same level when cells were grown in this compound and in diverse nitrogen sources, including NH4Cl, biguanide, and 1-N-methylbiguanide. This observation suggests a constitutive rather than tightly regulated gene expression. This research provides genomic and metabolic insights into metformin biodegradation by *Pseudomonas mendocina* strain MET, which can aid in the development of biotechnological applications to reduce the levels of metformin in the environment.

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Abstract 1737

Diadenosine Polyphosphatases of the NUDIX Hydrolase Superfamily in *M. tuberculosis* and *M. leprae*

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M. tuberculosis contains 11 potential Nudix hydrolases, and we are characterizing these enzymes as potential novel antibiotic targets. The diadenosine polyphosphatases (ApnAases)/mRNA decapping enzymes are a family of enzymes within the Nudix hydrolase superfamily. In *M. tuberculosis* there is the primary Nudix ApnAase and the secondary Nudix ApnAase. There are also orthologs of these two ApnAases in *M. leprae*. The diadenosine polyphosphatases from *Legionella pneumophilia* and *Bartonella bacilliformis* have been found to be important in each pathogen's ability to invade its host cells. It is of interest to know whether these enzymes act in the same way in *M. tuberculosis* and *M. leprae*. If they are all found to be involved in invasiveness and thus in virulence, then these enzymes could be novel antibiotic targets. We have cloned and overexpressed each protein and have subcloned each into a HisTag vector to optimize purification. The *M. tuberculosis* enzymes have been purified and characterized, and the primary ApnAase is in the process of being crystalized for structure determination. The *M. leprae* enzymes express too insolubly to purify and characterize, and thus we are working on increasing the expression of soluble protein so that we can study these enzymes as well; currently we know that they each have ApnAase activity (in the crude extract) above that of *E. coli* enzymes alone.

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Abstract 1745

Examining the Ability of Gut Bacteria to Metabolize Metformin

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Metformin is the drug most frequently prescribed for type 2 diabetes worldwide. This medication has been shown to alter the gut microbiome in diabetic patients, improving glucose metabolism. Metformin has been touted as having antiviral and anti-aging properties, making it a possible treatment option for other medical conditions. Studies have shown that 30% of patients who take metformin get sick and of those 5% discontinue the use of the drug. The mechanisms by which metformin alters the gut microbiome and causes side effects are poorly understood. The goal of this research was to investigate whether gut bacteria could metabolize metformin. If bacteria in the gut microbiome can degrade metformin, the degradation byproducts may alter the gut microbiota and cause patients to become ill. We hypothesized that bacteria in the human gut have proteins homologous to metformin degradation enzymes and can thus degrade metformin when it is used as a nitrogen source for growth. Bioinformatics analyses were conducted to identify proteins similar metformin degrading enzymes in the human gut microbiome. Two gene sequences, one from Blautia hydrogenotrophica and one from Lachnospiraceae symbiosum, were chosen for further study because they shared 20–30% sequence similarity to GbuAB, an enzyme that degrades metformin to guanylurea. The genes were cloned and over-expressed in *E. coli* to test these proteins'capacity to degrade metformin. Enzyme activity was tested by incubating the cell lysates with metformin, arginine, agmatine and 4-guanidino-butyric acid. HPLC analysis revealed no metformin degradation in the Lachno and Blautia lysates. A urea released assay showed these lysates degraded agmatine to urea with a specific activity of 10 umol/min/mg of protein. Growth studies to evaluate degradation of metformin when used as the nitrogen source were carried out using two different brands of commercial probiotics to model gut bacteria. The HPLC results show limited metformin degradation by these cultures. Taken together, these results indicate that gbuAB homologs from gut bacteria encode functional agmatinases rather than metformin-degrading enzymes, and that commercial probiotics have limited ability to degrade metformin; the absence of known metabolic intermediates suggests that the drug is not fully metabolized. Evidence from this work suggests that side effects experienced by patients taking metformin are not caused by byproducts of the drug's microbial degradation.

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ASBMB

Abstract 1759

Organic extract of medicinal lichen *Parmelia vagans* exhibits bactericidal activity against gram-positive and gram-negative bacteria

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Vladimir Bondarenko

Introduction: The World health organization deems resistant bacteria as one of the most severe threats to human health today. Given the implications resistant bacteria poses to both health and national security, searching for new methods and medications to overcome resistant bacteria is essential. Our research has shown a promising alternative to traditional antibiotics could come from the world of medicinal plants, specifically lichens, which are a group of composite organisms consisting of a fungus and a photosynthetic symbiote. While some of the secondary metabolites of lichens have been explored in the past to show antimicrobial activity, such as Usnic acid (UA) and Vulpinic acid (VA), most secondary metabolites have yet to be explored; this study will be the first report on the bactericidal properties of the secondary metabolites of lichen *Parmelia vagans*.

Methods: 50 g of dry lichens *Parmelia vagans* were extracted with 500 ml of acetone using the Soxhlet apparatus. Prepared extracts were filtered, and acetone was evaporated on the rotary evaporator. Antimicrobial activities of the extracts were tested using disc diffusion assay with known antibiotics as positive controls by measuring the diameter of the inhibition zone. Upon investigation, we discovered that the lichen extracts exhibited significant inhibitory activity against both gram-positive bacteria and Neisseria gonorrhea. To determine the mechanism(s) of inhibition, a bactericidal/bacteriostatic assay was performed using ampicillin and erythromycin as respective controls. After treatment with different concentrations of the lichen extract or antibiotics for 12 hours, 5 ul of bacterial culture was mixed with 150 ul of growth media and spread on a BHI agar petri dish and placed in an incubator at 37C for 12 hours. The bacterial colonies were counted, and the percent of the survived bacteria was calculated using untreated control.

Results: It was determined that extract activity was more akin to bactericidal activity against gram-positive and gram-negative bacteria at various concentrations. Antimicrobial activity was dose-dependent, though an exact ED50 will require more investigation. Additionally, when compared to the activity of UA and VA, the whole extract demonstrated alternative antimicrobial activity.

Discussion: These findings indicate that *Parmelia vagans* indeed exhibit relatively broad bactericidal activity in a concentration-dependent manner and could be useful in the future development of antimicrobial therapy. More studies are necessary to determine the mechanism of the bactericidal activity of the lichen extract.

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Abstract 1760

Nitric oxide sensing of *Vibrio cholerae* in biofilm formation and virulence

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Erik Yukl

Vibrio cholerae is the causative agent of cholera, a life-threatening secretory diarrhea which is transmitted by ingestion of contaminated food and water. The survival of *V. cholerae* in the aquatic environment and the human intestine relies partly on biofilm formation. Nitric oxide (NO) has emerged as an important signal regulating biofilm formation, and elevated levels of NO have been found upon infection with *V. cholerae* in the human intestinal lumen. The NO-responsive hemoproteins NosP and H-NOX act as NO sensors among various bacterial species and regulate biofilm formation in response to NO. It has been hypothesized that NO signaling by *V. cholerae* is critical for biofilm development and disease etiology. To evaluate this hypothesis, *V. cholerae* NO-sensing deletions strains ΔhnoX and ΔnosP were generated via homologous recombination and evaluated for their ability to grow under conditions of nitrosative and oxidative stress. There was no difference in growth rate between either mutant and the WT strain, suggesting that these gene products have redundant functions. We attempted to generate a ΔhnoX/ΔnosP double mutant strain, but this was unsuccessful after several attempts, suggesting that deletion of both genes may be lethal. Hence, one of the two genes was provided in trans on a pBAD18-kan plasmid under the control of the arabinose-inducible araBAD promoter prior to deleting the second gene. The resulting double mutant strain was designated as *V. cholerae* ΔhnoX/ΔnosP (phnoX). The expression of H-NOX from the pBAD plasmid was determined under various concentrations of arabinose and compared with growth and expression relative to WT and single mutant strains. These experiments show promise for determining the independent functions of these apparently redundant NO sensors and determining how important their NO-sensing functions are for *V. cholerae* survival, persistence and pathogenesis.

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Abstract 1761

Using gut microbiome analysis and machine learning approach for early cancer identification and treatments

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Background: Cancer is the second leading cause of death in the world. Unfortunately, survival rates for cancer patients have stayed poor since the 1970s. Thus, there is an unmet need to identify novel strategies for early cancer detection and prevention. Recent studies suggest that the gut microbiome including bacteria may have a role in cancer initiation and progression. These bacteria play a vital role in maintaining homeostasis in the body. An imbalance in the bacterial composition may cause diseases including cancer. This evidence led us to hypothesize that the changes in the composition of the gut microbiome can be used as a biomarker for early cancer detection. To test this hypothesis, we focused on analyzing microbial community of colorectal and blood cancers, which have poor survival rates.

Methods and Results: To analyze the association of cancer with microbiome, we used published microbiome dataset of leukemic and healthy individuals that was collected by sequencing stool samples (Oliver et al 2022). We identified that specific bacteria are either abundant, such as Faecalibacterium (1.17 fold), or reduced, such as Clostridium (0.32 fold, p value = 0.0029), in leukemia patients compared to healthy controls. We observed similar correlations by analyzing the published colorectal cancer data (Flemer et al., 2017) where Prevotella increased 3.75 fold and Escherichia/Shigella increased 4.0 fold in colorectal cancer while Blautia is reduced compared to healthy controls. Faecalibacterium, which is abundant in leukemia, is reduced in colorectal cancer. Further, we found that treatment of leukemia patients with specific inhibitors normalized the abundance of these bacteria to the level of healthy patients. To improve analysis efficiency, I applied machine learning algorithms to predict the presence of cancer in patients based on the composition of bacteria, age, gender, and symptoms. Among the tested algorithms, the decision tree and Random Forest both had very high false-negative rates, with 45.5% and 40% respectively, as well as having low sensitivities, with 50% and 60% respectively. Support Vector Machine had a suboptimal false-negative rate with 28.6% and good sensitivity of 80%. Finally, the Neural network had a good false-negative rate with 16.7% and great sensitivity of 90%. Overall, our results suggest that different cancers have the abundance of specific bacteria that can be used as biomarkers for cancer detection.

Conclusion: We demonstrate that the increased abundance of certain bacteria in the microbiome can be used as a biomarker for early cancer detection. Our data set was small for this project, but the machine learning approaches can be used to further predict the cancer These are clinically relevant findings as a microbiome test would be non-invasive and easily accessible that can be performed at a routine checkup.

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ASBMB

Abstract 1813

Investigating synergy between DesHDAP2, a novel histone-derived peptide, and different antibiotics

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In response to the drug-resistance crisis caused by the overuse of antibiotics, antimicrobial peptides (AMPs) have become a viable alternative treatment. Moreover, when combining AMPs with conventional antibiotics in a cocktail therapy, the two types of therapeutics can be synergetic. Previous work in the Elmore Lab designed a family of novel histone-derived antimicrobial peptides, DesHDAP1-3. The Elmore Lab previously tested synergy with the AMPs DesHDAP1 and the naturally occurring peptide buforin II (BF2), because they were both highly effective against bacteria when delivered alone. DesHDAP2, on the other hand, has been shown to be less potent on its own but has shown promise in preliminary cocktail therapy studies. In this study, we tested the synergy between DesHDAP2 and an array of antibiotics with different mechanisms of action (tetracycline, kanamycin, levofloxacin, rimpacilin, and cephalexin) against two bacterial strains, *E. coli* and *B. subtilis*. DesHDAP2 proved to be synergistic only with tetracycline and additive with kanamycin while other conventional antibiotics presented an indifferent effect. These results are interesting since the most synergistic combinations arose between antibiotics with an internal bacterial target and the DesHDAP2 peptide that permeabilizes the cell membrane.

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Abstract 1815

Development of Whole Cell Biosensor Systems for Positional Biosensing in Bacterial Biofilms

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Current microbial whole cell biosensors (MWCB) systems provide sensitive and selective detection of soluble analyte concentrations in a liquid sample. While valuable, these biosensors do not give any other information beyond the analyte concentration such as the time- and position dependent communication in response to the analyte within the microbial community. To better understand bacterial communication in natural living conditions we have measured the response of MWCB in response to analytes in bacterial lawns and biofilms. In these Positional Biosensors (PBS), the analyte activates a promoter regulating an autonomous bioluminescent cassette (e.g., LuxCDABE from Photorhabdus luminescens, or LuxCDABEG from Aliivibrio fischeri) and the response to the analyte is measured in a time- and position-dependent manner by an *in vivo* imaging system (IVIS). To elucidate the mechanisms involved in the analyte-specific response, we have used *Escherichia coli* strains overexpressing regulatory factors or carrying deletions. We have tested several PBS developed in our laboratory for the monitoring of analytes relevant for bacterial communication, detoxification and biofilm formation including quorum sensing molecules (QSM) and environmental chemicals. Interestingly, some of these PBS not only show a time and position dependent response to their bona-fide analytes, but also to other stimuli such as antibiotics, metabolites, oxidants, micronutrients, and toxic molecules. The response to these stimuli may reflect increased communication in the bacterial biofilm in defense of the stressors, possibly enhancing resistance and increasing survival.

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Abstract 1855

Lacritin bactericidal peptide 'N-104' interrupts spermidine and ferrous iron uptake in *P. aeruginosa*, PA14

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Fatemeh Norouzi, Michael Lemke, Thurl Harris, Gordon Laurie

Our unbiased screen of the *E. coli* Keio collection of 3985 nonessential gene knockouts followed by validation experiments with the human opportunistic pathogen, *P. aeruginosa* PA14 have revealed the important roles of the inner-membrane transporters PotH (polyamines) and FeoB (ferrous iron) in the bactericidal mechanism of human tear lacritin peptide 'N-104'. Furthermore, N-104 directly binds PotH and FeoB under physiological salt concentrations. Does N-104's interaction with either protein interrupt its function? In the polyamine transport assay, PA14 cells were grown in M9 minimal salts (suppl. with 0.4% D-glucose) for 48 h. 100 µl of PA14 samples (0.8 × 108 cfu) were washed with 0.9% NaCl and resuspended in 0.1 × PBS before incubation without or with 10 µM 1,4-14C-putrescine (14C-Put) or 1,4-14C-spermidine (14C-Spd) for 15 min at 35°C. In pretreated samples with N-104 or N-80/C-25 (negative control peptide), cells were incubated with 10 µM of either peptide for 45 min at 35°C prior to polyamine treatment. Cells were washed four times with PBS to remove excess polyamines and peptides. Pellets were transferred to 4 ml of scintillation liquid to measure intracellular 14C-polyamines contents using standard curves for each molecule. To probe iron uptake, PA14's intracellular iron was first depleted from overnight cultures (5 × 109 cfu) using 1 mM 2,2'-bipyridyl. Cells were washed with 0.9% NaCl and resuspended in 0.1 × PBS before incubation with 100 µM FeSO4 (suppl. with 13 mM ascorbic acid) for 15 min at 35°C. Washed cells were incubated with 20 µl of iron-releasing reagent (HCl and KMnO4) for 30 min at 80°C. Samples were cooled down to room temperature before incubation with ferrozine reagent (suppl. with neocuproine, ammonium acetate, and ascorbic acid) for 30 min at room temperature. Samples were spined and absorption of 80 µl of supernatant was recorded at 565 nm. In pretreated samples with N-104 or N-80/C-25, cells were incubated with 10 or 25 µM of each peptide for 45 min at 35°C prior to iron treatment. Our results showed that (0.64 ± 0.20) × 106 14C-Spd molecules were transported to each PA14 cell that was two times higher than 14C-Put uptake ((0.33 ± 0.06) × 106 molecules per cell). Spd content in *P. aeruginosa* cells is reported to be roughly 10 times higher than Put content (3 mM vs. 0.3 mM). 10 µM N-104 (but not the control peptide) reduced 14C-Spd uptake by 30%. Given that PA14 cells grown in M9 growth medium are more virulent than the cells grown in LB broth medium (with 8-fold increase in N-104's MIC value), and that N-104 only interacts with PotH subunit of the polyamine transporter channel (Pot- channel), the 30% reduction in 14C-Spd uptake was still significant. Moreover, iron uptake in PA14 cells ((2.49 ± 0.19) × 106 ions per cell) was reduced by 25 µM N-104 for up to 61%. 47% reduction in iron uptake was observed for 10 µM N-104 treatment. FeoB is a well-recognized virulent protein in *P. aeruginosa*, *E. coli*, *H. pylori*, and some other species. Moreover, polyamines are the most abundant and versatile molecules in bacterial cells that play roles in several physiological functions such as biosynthesis of the natural products. We argue that N-104-induced changes in molecular uptake of iron or Spd might trigger programmed cell death. The joint iron and polyamine transporter blockage by N-104 as a natural peptide component of human tears uncovers a novel method of bacterial cell death that can be used as a new antibiotic strategy. Note that lacritin is also present in saliva, plasma, and lung lavage.

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ASBMB

Abstract 1885

Are the Arginine Kinase functions found in *Myxococcus xanthus* conserved in a sister species, *Myxococcus macrosporus*?

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Dean Fraga, Jorge Gonzales Virto

Phosphagen Kinases (PK) catalyze the reversible phosphorylation of the guanidino group to buffer energy in all animal species and some protozoa. Recently, genomic sequencing of bacterial species revealed that a few contain arginine kinases (AK) that phosphorylate arginine. Based on phylogenetic analyses, it was found that the most closely related sister bacterial species are missing AK homologs, suggesting that these bacterial AK (bAK) are derived independently from horizontal gene transfer (HGT). Among these bacterial species with AKs, the myxococcus family has several with AKs that appears derived from an ancestral HGT event based upon synteny between the different species. Two of these, *Myxococcus xanthus* and *Myxococcus macrosporus*, were selected for this study. A previous study using *M. xanthus* found unique roles that AK plays in social and physiological behaviors, such as kin discrimination and fruiting body formation. In this study, we investigate the role that AK plays in *M. macrosporus* by knocking out the AK gene, ark, and compare it to the mxAK (*M. xanthus* AK) using kin discrimination, sporulation, and stress assay. Results from this study will help us determine which roles the AK evolved subsequent to HGT but prior to speciation and which were later derived in the two species.

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Abstract 1900

Plipastatin contributes to copper homeostasis in *B. subtilis*

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Pete Chandrangsu, Maya Addison

Antimicrobial resistance is a large problem today as microbes find new ways to evade antimicrobials. Thus, it is necessary to understand how microbes can evolve to escape these systems. Here, we use experimental evolution, genetics, and biochemistry to understand how the model Gram-positive bacterium, *Bacillus subtilis*, develops resistance to copper, a known antimicrobial. Copper is known to cause damage to cells through contact killing and has been used for its antimicrobial properties for centuries, dating back to early Egyptian texts. Genome sequencing revealed that all mutants that survived on high copper concentrations had a mutation in the ppsB gene, leading to a loss of plipastatin synthetase. Plipastatin is an antibacterial compound and helps protect the bacterium from competing bacteria and fungi. In conclusion, loss of ppsB leads to a greater ability of *B. subtilis* to grow in increasing copper concentrations and future research will evaluate whether plipastatin is shuttling copper into the cell. These findings may be important in potentially identifying plipastatin as a chalkophore.

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Abstract 1903

Nramp Metal Transporter Gene Contributes to Copper Resistance in *Staphylococcus epidermidis*

Emily Nguyen, *Claremont McKenna College*

Pete Chandrangsu

The ongoing battle against antibiotic resistance implores us to look deeper into traditional medicines. Copper is documented as an ancient Egyptian treatment for a variety of ailments in the Smith Papyrus and the Ebers Papyrus. *Staphylococcus epidermidis* symbiotically lives on human skin and mucus, but is an opportunistic pathogen once it invades the human body. These invasions primarily occur via medical and prosthetic devices, making it one of the leading causes of nosocomial infections. To understand how *S. epidermidis* evolves resistance to the antimicrobial effects of copper, multiple generations of *S. epidermidis* were grown in increasing concentrations of Cu. The initial MIC was nearly doubled from 3–4 mM Cu to 5–6 mM Cu. Eight colonies of the evolved strain underwent genome sequencing to determine the potential mutations that caused their copper resistance. All colonies were found to have mutation in a putative Nramp family divalent metal transporter gene. Future studies will focus on creating an in-frame clean deletion to investigate the relationship between the mutation and *S. epidermidis*' resistance to copper. Our findings will contribute to the discovery of novel antibiotic treatments to combat Staphylococcal infections.

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Abstract 1976

Developing a New CRISPRi Toolkit for Marine Bacteria

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Amanda Alker, Nicholas Shikuma

The genetic tools to study host-microbe interactions and their underlying processes for marine bacteria are currently limited. To diversify the tools available for genetic manipulation of marine bacteria, we set out to expand a genetic toolkit and develop new parts that broaden compatibility. To develop and test our new tools, we used the marine bacterium *Pseudoalteromonas luteoviolacea* that produces a contractile injection system, called Metamorphosis-Associated Contractile Structures (MACs), to induce metamorphosis of the tubeworm *Hydroides elegans*. We hypothesize that by using CRISPRi techniques to target metamorphosis-inducing genes in *P. luteoviolacea*, we will be able to knockdown gene expression in the production of MACs. CRISPRi Golden Gate Assembly (GGA) was utilized to assemble different DNA fragments (pBTK) into a plasmid that targets the *P. luteoviolacia* metamorphosis-associated gene. The assembled CRISPRi plasmid was then conjugated into *P. luteoviolecea*. To confirm the successful knockdown and its subsequent effect on tubeworms, we used metamorphosis as the biological readout in a biofilm metamorphosis assay involving *H. elegans* and our conjugated marine bacteria. The genomic components of our plasmid toolkit allow for its broader use in a variety of different marine bacteria. This project provides a proof of concept that we can manipulate the phenotypes of marine microbes using modular genetic engineering techniques. Manipulation of marine bacteria has the potential to benefit industries as diverse as aquaculture, biomedicine, and biomanufacturing. Future biotechnology in gene manipulation of marine bacteria can be carried out since different genes reflect different functions and marine bacteria remain an untapped resource for biotechnology.

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ASBMB

Abstract 1988

Investigating the effect of polyamine starvation on *Leishmania* parasite's nuclear DNA and mitochondria

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A better understanding of *Leishmania* biology is vital for the development of much needed new therapeutic strategies. Our research addresses this urgent need by focusing on the role of polyamines for growth and survival of *Leishmania* parasites. The polyamine pathway has already been validated as a drug target in the related parasite, *Trypanosoma brucei*, and recent studies have highlighted their critical nature in *Leishmania*. The main polyamine biosynthetic enzymes in *Leishmania* are ornithine decarboxylase (ODC) and spermidine synthase (SPDSYN), which sequentially convert ornithine to the polyamines, putrescine and spermidine. The gene deletion mutants, *Δodc* and *Δspdsyn*, show significantly reduced infectivity phenotypes in mice, validating this pathway as a potential therapeutic target. Our *in vitro* studies revealed that the upstream metabolite putrescine is essential beyond its function as the precursor for spermidine synthesis. Furthermore, we observed that putrescine-depleted *Δodc* parasites immediately cease proliferation and die within two weeks. In contrast, putrescine-rich *Δspdsyn* mutants show an intermediate growth phenotype and enter into a quiescent-like state with cell death occurring after six weeks. Together these studies led to the key finding that putrescine plays previously unrecognized yet vital roles for proliferation and survival. We are now in the process of elucidating the functions of putrescine for these key processes. Preliminary data suggests that putrescine may be essential for DNA stability and mitochondrial health. A nuclear fluorescent DNA stain, NucBlue, was used to assess cellular DNA content under the microscope. Mitochondrial health was evaluated by using JC-1, a fluorescent stain whose color depends on mitochondrial membrane potential, and by utilizing a resazurin conversion assay, which measures mitochondrial metabolism. NucBlue staining showed decreased amounts of nuclear DNA after 8 days of starvation in the *Δodc* mutants, compared to wild type and polyamine supplemented cells. This suggests that putrescine depleted *Δodc* cells undergo nuclear DNA degradation. JC-1 staining demonstrated that the *Δspdsyn* supplemented cells were the only cell line containing healthy mitochondria, thus indicating that spermidine is vital for mitochondrial function. To further test this observation, a resazurin assay was conducted looking at the conversion rate of resazurin to resorufin. The *Δspdsyn* parasites supplemented with spermidine showed the highest rate of resazurin conversion, supporting the observation that spermidine is important for mitochondrial function. This study suggests that putrescine is vital for the integrity of nuclear DNA of *Leishmania* parasites, while spermidine is vital for mitochondria function.

This work is supported in part by Grant R15AI151980 from the National Institute of Allergy and Infectious Diseases.

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Abstract 2002

Investigating comorbidity of hereditary spastic paraplegia and toxoplasmosis

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The diverse set of neurodegenerative disorders collectively known as hereditary spastic paraplegias (HSPs) lead to a length-dependent axonopathy within the corticospinal tract, causing lower limb spasticity and coordination deficits in patients. HSPs are heritable and have been linked to a variety of mutations, including the p.R106C mutation in TRK-fused gene (TFG), a protein involved in the secretory pathway, which facilitates transport from the endoplasmic reticulum (ER) to the ER-Golgi intermediate compartment (ERGIC). Most neurodegenerative diseases, including HSPs, present with generally heterogeneous onset and severity, but the mechanisms underlying this heterogeneity remain unclear. Using CRISPR/Cas9-mediated genome editing, we have introduced the TFG p.R106C mutation into Sprague Dawley rats and demonstrated that these animals develop progressive hindlimb dysfunction, thinning of the corpus callosum, and enlargement of the lateral ventricles consistent with disease phenotypes observed in HSP patients. We have further begun to investigate the impact of infectious disease on neurodegenerative disease course by infecting a group of animals harboring the TFG p.R106C mutation with the parasite *Toxoplasma gondii*, which can independently result in coordination deficits, particularly of the lower limbs. *T. gondii* is able to infect most warm-blooded mammals, including humans; 30–50% of the global population is estimated to carry a chronic *T. gondii* infection. During *T. gondii* chronic infection, cysts form in neurons, likely disrupting axonal firing. Our preliminary data indicate that *T. gondii* infection in our animal model of HSP results in an accelerated disease course, suggesting that infectious disease may contribute to disease heterogeneity in patients suffering from neurodegenerative disorders.

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Abstract 2008

dishevelled gene expression is associated with Rift Valley fever virus susceptibility in *Aedes aegypti* mosquitoes

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Rift Valley fever virus (RVFV) is a zoonotic mosquito-borne virus that infects livestock and humans. In humans, RVFV causes fever-like symptoms, birth defects, and sometimes death. We sought to better understand mosquito cell signaling responses late in RVFV MP-12 infection through analysis of differential gene expression in the mosquito vectors, *Aedes aegpyti* (Aae) and *Culex tarsalis* (Cxt). Cxt are more susceptible to MP-12 viral infection, with 100% of the samples having viral RNA present 14 dpi, compared to 61.7% in Aae. Therefore, we expected that differences in gene expression levels may also occur. Expression levels of five transcripts were analyzed using RT-qPCR: Armadillo (ARM), Frizzled2 (FZ2), and Dishevelled (DSH), of the Wingless pathway, Puckered (PCK), of the c-Jun N-terminal Kinase (JNK) pathway, and Domeless (DOME), of the Janus Kinase/Signal Transducers and Activators of Transcription (JAK/STAT) pathway. Transcript expression levels in RVFV MP-12 infected mosquitoes were compared to uninfected controls. For Aae, it was found that DSH, PCK, ARM, and DOME showed significant expression level differences (t-test, $p < 0.05$). For Cxt, DSH, FZ2, PCK, and DOME showed significant differences, while ARM did not (t-test, $p < 0.05$). These data suggest RVFV infection results in prolonged changes to cell signaling pathways in both species. In addition, Aae showed significant inverse correlation between DSH log2 fold-change and virus log-copy numbers. Therefore, DSH may play a significant role in mosquito anti-viral immunity. In an upcoming experiment, viral loads will be measured following DSH silencing at 3 and 7 dpi post viral challenge. These results point to the significance of cell signaling pathways in vector competence and provide a foundation for future work to define the signaling pathways that determine mosquito susceptibility to arboviruses.

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Abstract 2035

Towards bacterial expression of helminth mitochondrial aminoacyl-tRNA synthetases

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The WHO classifies helminths as "Neglected Tropical Diseases" because the parasitic worms often infect people who live near the equator. These species, including hookworms, roundworms, and flatworms, are estimated to affect about a quarter of the world's population – particularly in the developing world. Aminoacyl-tRNA synthetases (ARSs) are crucial enzymes for protein biosynthesis in any organism. Since ARSs are essential enzymes, inhibiting parasitic ARS is a potential strategy for treatment of infection. Two helminth mitochondrial ARS, specific for alanine and arginine, are significantly different from their human homologs. Bacterial expression of mitochondrial proteins often requires removal of the mitochondrial targeting tag (MTS). We used sequence and structure prediction algorithms to identify potential MTS sequences in mtAlaRS and mtArgRS from three helminths species, and constructed bacterial expression systems in which putative MTS sequences were removed in order to optimize protein production for future work on enzymatic characterization.

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Abstract 2067

Preventing Post-coital Urinary Tract Infections with Lactobacillus-Impregnated Hydrocolloid

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Urinary tract infections, especially those occurring post-coital, are one of the most common infections requiring prescribed antibiotics. Over half of all women will experience a UTI within their lifetime, with many having a recurrent infection within the same year. Uropathogens, or bacteria that cause urethritis and bladder infections, are constantly evolving to become resistant to the most common antibiotics used to treat UTIs. As a result, treatment-resistant infections are becoming more common. In order to address this crisis in healthcare, a physical barrier was created for the urethra using hydrocolloid material impregnated with Lactobacillus bacteria to develop a viable alternative to antibiotics for post-coital prophylaxis. Lactobacillus is a beneficial strain of bacteria found in the vaginal canal that has a metabolic byproduct of hydrogen peroxide, which effectively kills uropathogens. Common uropathogenic agents, *Escherichia coli*, *Staphylococcus saprophyticus*, and *Candida*, were cultured and tested against hydrocolloid impregnated with probiotic bacteria *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus crispatus*, and *Lactobacillus gasseri*. Bioassays measuring minimum inhibitory concentrations (MICs) were used to examine the efficacy of these beneficial bacteria. The observed data gives insight into understanding how the vaginal microbiota provides protection against urinary tract infections. Additionally, the product of this study would be used to prevent bacterial colonization of the urethra during intercourse. Creating novel ways of prevention instead of utilizing antibiotics after infection sets in is vital in fighting against the ever-growing crisis of antibiotic-resistant bacteria.

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Abstract 2068

Combinatorial Antimicrobial Effects of Imidazolium-based Ionic Liquids and Antibiotics on Model Pathogenic Microorganisms

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Ionic Liquids (IL) are a unique class of molten salts, of which specific formulations have been shown to independently exhibit antimicrobial properties. Several recent studies have highlighted the ability of ILs to form micelles, permeabilize the extracellular membrane, and ultimately destabilize cellular structure which at high concentrations of IL can lead to cell death. Moreover, while these membrane-destabilizing properties are both effective and lethal in organisms ranging from bacteria to human cells at high concentrations, at lower concentrations, membrane destabilization may lead to improvements in drug delivery for combinatorial therapies. The principle aim of this study is to identify a synergistic relationship between ILs, 1-n-Hexyl-3-methylimidazolium chloride (HMIM[Cl]) and 1-Methyl-3-n-octylimidazolium chloride (OMIM[Cl]), and a broad set of antibiotics testing the hypothesis that in a combinatory setting there should be improved antibiotic efficacy against model pathogenic microorganisms. Using several complementary assays to identify the combined effects of IL + antibiotic treatment, including Kirby-Bauer tests and minimum inhibitory concentrations (MIC) assays to establish antimicrobial effects, as well as flow cytometry to evaluate permeability, we convincingly demonstrate that at low concentrations, the ILs tested in this study are capable of improving the effectiveness of current low-efficacy antimicrobial compounds. Future work stemming from these results will aim to identify the cytotoxic effects of these combined treatments for use in topical treatments and ultimately the potential as a viable treatment to combat antimicrobial resistance microorganisms.

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Abstract 2072

Novel lead compounds demonstrating anti-biofilm properties in *Staphylococcus aureus* may act as Nor-A efflux pump inhibitors

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Numerous studies have shown that bacteria quantify their density and collectively carry out a certain response with a communication mechanism known as quorum sensing. Biofilms are a type of virulence factor that forms once a bacterial quorum is reached within the host, preventing the host's immune system from detecting and ultimately eradicating these pathogenic bacteria. Efflux pumps may also be involved in biofilm production. These pumps are transport proteins that are responsible for the extrusion of various substances, including toxins, quorum sensing molecules, and biofilm components. The increased activity of efflux pumps has allowed antibiotic-resistant bacteria to increase their survival rates. This study tests certain compounds that were designed to resemble quorum sensing signaling molecules to inhibit biofilm formation. Since these compounds also have structural homology with known Nor-A efflux pump inhibitors (EPI) in *S. aureus*, we tested their activity on this pump. Fourteen of our previously synthesized compounds share this structural homology, with five of them revealing significant biofilm reduction in SA-1199 (wildtype) and SA-1199B (Nor-A overexpressor) but not in SA-K1758 (Nor-A knockout). This indicates that the compounds function may be in targeting the Nor-A pump rather than as competitive inhibitors at the receptor sites of the *S. aureus*. The conjugated aromatic ring with alternating electron-withdrawing and donating groups on the ortho and para positions appear to affect the inhibitory characteristics of these drugs. Developing a cocktail of drugs that curtail quorum sensing and inhibit efflux pump activity can maximize biofilm inhibition in *S. aureus*.

The Mercer University Quality Enhancement Plan: Research that Reaches Out, the Mercer University Chemistry Department and the Mercer University Biology Department.

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Abstract 2085

Targeted Proteomics to Identify DNA Replication and Repair Proteins in the Malaria Parasite Plastid

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Malaria is an ancient disease caused by unicellular protists of the genus Plasmodium, with *P. falciparum* being responsible for most cases of severe disease and deaths in humans. Plasmodium species contain a relict plastid called the apicoplast (AP) that is critical to parasite metabolism and an important drug target. Like other endosymbiotic organelles, the apicoplast contains its own genome that must be replicated and repaired for parasite survival. The single AP DNA polymerase (apPOL) that replicates and repairs the AP genome is encoded by a nuclear gene called the Plastidic DNA Replication/Repair Enzyme Complex (PREX). PREX is expressed as a polyprotein fusion (Primase-Helicase-Polymerase) that is cleaved into its respective components upon import into AP lumen. There are three gaps in knowledge associated with PREX biology and function that we are currently investigating; (1) the protease(s) that cleave PREX polyprotein into its individual components have not been identified; (2) the apPOL processivity factor required for processive deoxynucleotide insertion during DNA replication is unknown; and (3) despite efforts from both bioinformatics and organelle-wide proximity labeling, several expected DNA repair pathway components have not been identified. We have developed a targeted proximity-labeling approach that fuses a promiscuous biotin ligase to known AP DNA replication/repair enzymes such as apPOL to identify proteins of interest that are associated with PREX biology and function. We utilized a CRISPR-Cpf1 gene editing strategy to generate a parasite line bearing a DiCre-recombinase inducible C-terminal TurboID fusion to the endogenous apPOL gene. To establish a labeling control, an AP-targeted version of TurboID was integrated into a benign locus of same cell line and conditionally expressed under the same promoter as apPOL. Results from gDNA PCR, Western blot, and indirect fluorescent assays demonstrate successful editing of each locus and inducible labeling of apicoplast proteins. Preliminary results from proteomics suggest apPOL-TurboID successfully labels AP proteins in a targeted manner. The AP labeling control enabled a stringent selection process that produced a high confidence list of twelve candidates whose functions are associated with pathways of interest. Efforts to identify unknown proteins through structural homology searches and future experiments planned to validate the high confidence candidates are discussed.

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Abstract 2105

Structural and Biochemical Analysis of Phosphoethanolamine Methyltransferase from the Pine Wilt Nematode *Bursaphelenchus xylophilus*

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Joseph Jez

In free-living and parasitic nematodes, the methylation of phosphoethanolamine to phosphocholine provides a key metabolite to sustain phospholipid biosynthesis for growth and development. Because the phosphoethanolamine methyltransferases (PMT) of nematodes are essential for normal growth and development, these enzymes are potential targets of inhibitor design. The pine wilt nematode (*Bursaphelenchus xylophilus*) causes extensive damage to trees used for lumber and paper in Asia. As a first step toward testing BxPMT1 as a potential nematicide target, we determined the 2.05 Å resolution x-ray crystal structure of the enzyme as a dead-end complex with phosphoethanolamine and S-adenosylhomo cysteine. The three-dimensional structure of BxPMT1 served as a template for site-directed mutagenesis to probe the contribution of active site residues to catalysis and phosphoethanolamine binding using steady-state kinetic analysis. Biochemical analysis of the mutants identifies key residues on the β1d-α6 loop (W123F, M126I, and Y127F) and β1d-α6 loop (S155A, S160A, H170A, T178 V, and Y180F) that form the phosphobase binding site and suggest that Tyr127 facilitates the methylation reaction in BxPMT1.

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Abstract 2109

Proteomic Changes of *Vibrio cholerae* in Response to Mannitol

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Vibrio cholerae is the causative agent responsible for cholera, an acute diarrheal disease that affects 3–5 million people each year. Biofilm formation is thought to be one strategy that *V. cholerae* employs to survive outside of a host. Our lab and others have shown that the naturally occurring carbon source mannitol is able to induce biofilm formation in *V. cholerae*. Therefore, we are interested in identifying the proteomic changes that occur upon the bacteria's exposure to mannitol. To address this objective, we used bioorthogonal noncanonical amino acid tagging to identify proteins that were newly synthesized by *V. cholerae* in response to exposure to mannitol. *V. cholerae* cell cultures were grown in minimal medium supplemented with mannose before being pelleted and resuspended in fresh minimal medium supplemented with mannitol. The noncanonical amino acid, L-azidohomoalanine (AHA), was added to the fresh mannitol medium and cultures were grown for an additional 45 minutes before being lysed. The newly synthesized proteins were tagged with biotin. Through western blot analysis, we confirmed the incorporation of AHA, as well as the tagging of proteins with biotin. Subsequently, the biotinylated proteins were isolated from the rest of the proteome. Eluted samples were analyzed via LC/MS/MS. Preliminary results reported a total of 217 proteins identified, 197 which were *V. cholerae* proteins. The identified proteins included a variety of molecular functions including structural activity and signaling activity. These proteins most likely naturally occur at higher abundances, therefore next steps include increasing protein yield and confidence of identified proteins to observe less abundant proteins. Being able to identify the proteomic changes that help *V. cholerae* persist in aquatic environments will help lead to the development of different strategies for better control and prevention of cholera disease.

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Abstract 2164

Swarming Behavior of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a ubiquitous organism that is an opportunistic pathogen responsible for many infections, such as those of patients with burns, chronic infections, and cystic fibrosis. This organism has intrinsic and acquired resistance mechanisms against antibiotics and is a frequent causative agent of nosocomial infections. A process central to the pathogenesis of *P. aeruginosa* is its ability to form communities known as biofilms where it exhibits social behaviors and quorum sensing. Relevant to social behaviors and biofilm are different types of motilities displayed by *P. aeruginosa*. Three types of motilities in *P. aeruginosa* are swimming, swarming, and twitching. Swimming is single bacterium movement due to a single polar flagellum that does not require quorum sensing and occurs in low-viscosity conditions (e.g. 0–0.3% liquid-agar). Swarming is linked with biofilm formation by its coordinated multicellular movement that appears to require two polar flagella or an alternative motor and occurs on a semi-solid surface (e.g. 0.5–1% agar). Twitching is movement that is powered by the extension, tethering, and retraction of polar pili and occurs on a moist solid surface (e.g. 1.5% agar). Given our interest in biofilm and its importance in antibiotic resistance and relevance to clinical infections, we pursued studies on swarming of *P. aeruginosa*. In reported studies, phytochemicals such as curcumin have been shown to reduce or inhibit swarming ability. It is therefore important to consider treatments that are alternatives to antibiotic use. For example, phytochemicals that target swarming may serve as biofilm inhibitors that are effective, less costly, and relatively non-toxic. In this study we investigated various swarming protocols reported in the literature to identify an optimal procedure to serve as a screening assay for anti-swarming effect. Procedures assessed included variations in type of media used, agar percent in the media, size of the agar plate, use of microwells and others, as we searched for an optimal swarming/anti-swarming quantitative procedure. Important components for a screening procedure included (i) inoculation of bacteria into agarose media, either by inoculating needle or direct pipetting into the agar and (ii) use of larger plates that provide a larger swarming distance thereby making it optimal for discerning swarm inhibition, enhancement, or no effect. Further studies focus on refinement of swarming assays and assessment of phytochemicals for anti-swarming potential.

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ASBMB

Abstract 2177

Determination and Comparison of the Total Flavonoid Content and Antibacterial Activity of Three *Acacia rigidula* Benth Extracts

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Ruby Ynalvez

The excessive use of antibiotics has led to the rapid development of drug resistant bacteria creating a severe public health concern. The development of new drug treatments is being outpaced by emerging resistant bacteria placing great importance for discovering novel sources of antibacterial compounds. Plants have been used in traditional and folk medicine for centuries making plants prime candidates for discovering antimicrobial compounds due to the vast variety of secondary metabolites, namely flavonoids. Recent studies have begun to uncover the potential of the South Texas plant *Acacia rigidula*, but there is a lack of literature and consensus on the optimal extraction method of secondary metabolites and their corresponding antimicrobial activity. In this study we report the antimicrobial activity of three different solvent extracts of *Acacia rigidula* benth, with an aim to determine the optimum extraction solvent for antibacterial compounds. Additionally, we aim to isolate flavonoids from *A. rigidula* and evaluate the antibacterial activity of the isolates. Plant leaves were subjected to Soxhlet extraction using 50% ethanol, water: acetone: methanol (WAM) (1:1:3), or 80% methanol as the solvent. Flavonoid isolation occurred by low pressure column chromatography using Sephadex LH-20 and methanol as the eluent. Qualitative analysis for the total flavonoid content (TFC) was measured by the aluminum trichloride assay using quercetin as the standard. A disc diffusion assay (DDA) was employed using 5 bacteria to determine the antibacterial activity of the extracts by measuring the mean zone of inhibition (mZOI). Statistical analysis revealed no difference ($p > 0.05$) in the TFC values between 50% EtOH, WAM, and 80% MeOH. Quantitative TFC analysis measured the TFC concentrations to be 104.51 mg QE/g, 108.42 mg QE/g, and 109.50 mg QE/g for 50% EtOH, WAM, and 80% MeOH respectively. The DDA analysis showed *S. auricularis* (mZOI: 22.00 mm, 22.81 mm,22.82 mm) and *S. epidermidis* (mZOI: 18.03 mm, 18.00 mm, 17.56 mm) having susceptibility to 50% EtOH, WAM, and 80% MeOH respectively with each extract showing significant difference ($p < 0.05$) to the positive control antibiotic for *S. auricularis*. *M. luteus* and *E. coli* showed intermediate susceptibility and resistance to the three solvent extracts respectively. Preliminary DDA results of the flavonoid isolates shows promising antibacterial activity of 8 main fractions generated by column chromatography and analyzed by TLC. The results of the DDA of the three solvent extracts and flavonoid isolate provide evidence that *A. rigidula* leaves have antimicrobial activity and warrant the additional analysis of the extracts against highly virulent and antibiotic resistant bacteria.

College of Arts and Sciences, Texas A&M International University Graduate School, Texas A&M International University.

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Abstract 2219

Towards a Structural and Atomic Mechanism for NIS Synthetases

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Recently, the family of adenylating enzymes was expanded to include two subfamilies of siderophore synthetases. One of them, the NIS Synthetases, is an excellent target for structure-based antibiotic drug design due to its novel structure and function, and association with virulence. The adenylating NIS synthetases are understudied, with only four different proteins structurally characterized of a family wide enough to have three subtypes and iterative behavior. Additionally, a full complex of protein, cofactor, carboxylate and amine substrates has been elusive. Biochemical characterization too, has lagged, though three different assays have been published, their range of reported values is inconsistent. The desferrioxamine siderophores (dfoD, G, E and B) are all made by Streptomyces bacteria through the desferrioxamine A, B, C and D pathway (DesABCD). DesD, a type C NIS synthetase, iteratively catalyzes the last three bonds made in this pathway to sequentially create all of the known siderophores in *S. coelicolor*, and end with the macrocyclized dfoE, a circular trimer of N-hydroxy, N-succinyl cadaverine. We have solved the structure of DesD in apo and ATP-bound forms, as well as explored the structure of several active site variants. Additionally, a label-free, ITC-based kinetics assay has been instrumental in describing the critical catalytic residues and their role in the proposed two-step mechanism. We present a proposal for the two-proton dependance mechanism of bond formation, as well as a pH profile and structural information to support it. Additionally, we present preliminary binding studies to guide future attempts at complex crystallize and to further explore the structural mechanism. This basic science foundation will be utilized in the future for structure-based drug design and testing of novel antibiotics.

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Abstract 2233

Structure-based Antiviral Design against HTLV-1 Protease

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Human T-cell leukemia virus type-1 (HTLV-1) is the first-discovered oncogenic human retrovirus and has infected over 20 million people worldwide. HTLV-1 infection causes adult T-cell leukaemia/lymphoma (ATL) and various inflammatory diseases in 5–10% of patients. Poor patient outcomes highlight the dire need for effective treatments, but there are currently no vaccines or direct-acting antivirals (DAA) for HTLV-1 infection. Similar to the closely related HIV-1 protease, which is the target of several FDA-approved DAAs, HTLV-1 protease is a homodimeric aspartyl protease essential for the cleavage of functional proteins from viral polyproteins. Despite this similarity, HIV-1 protease inhibitors show poor inhibition of HTLV-1 protease. We hypothesized that HIV-1 protease inhibitors, particularly the most recently FDA-approved and most potent darunavir, can be modified to target the protease of HTLV-1 toward potent DAAs against HTLV-1 infections. We have designed and implemented a FRET-based peptide cleavage assay to assess the potency of HIV-1 protease inhibitors and their novel analogs against HTLV-1 protease, which we have designed and synthesized in-house. Our crystal structures of these inhibitors bound to HTLV-1 protease granted insights into inhibitor binding interactions that can be improved through moiety modifications at the P1, P1' and P2' sites. Informed by the crystal structure analysis, we have designed transition-state analog inhibitors with low nM potency against HTLV-1 protease. PU6, our inhibitor with the best potency toward HTLV-1 protease with over 100-fold improvement over darunavir, shows a mode of binding at the S1' site that is distinct from HIV-1 protease. This novel binding mode of PU6 not only demonstrates the necessity for further investigation of HTLV-1 protease cocrystal structures, but also informs the next iteration of design modifications at the P1' site to further increase potency toward generating effective DAAs against HTLV-1.

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ASBMB

Abstract 2234

CD46 is a protein receptor for Human Adenovirus Type 64

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Adenoviruses are important gene delivery vectors and causative agents for a variety of human diseases such as the common cold and gastrointestinal infections. Human adenovirus type 64 (Ad64; formerly 19c) and type 37 are associated with epidemic keratoconjunctivitis. Based upon its high homology and similar disease tropism to Ad37, we hypothesized that Ad64 would have the same protein receptor, CD46 (membrane cofactor protein), as Ad37. We show that a recombinant Ad64 containing an enhanced Green Fluorescent Protein transgene (Ad64.eGFP) enters Chinese hamster ovary cells expressing human CD46 (CHO-CD46) on the surface. Entry into human cervical carcinoma (HeLa) cells is increased by the presence of calcium, but that increase can be blocked by an anti-CD46 antibody. Ad64.eGFP gene delivery into human conjunctival epithelial (HCjE) cells can also be blocked by soluble CD46, supporting our hypothesis that Ad64 uses CD46 as a protein receptor on the surface of target cells. CD46 expression on CHO-CD46, HeLa, and HCjE cells was confirmed by flow cytometry and/or western blotting. The identification of CD46 as a receptor for Ad64 makes Ad64 an attractive candidate for gene delivery into eye cells as well as other cells that express CD46.

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Abstract 2254

Inhibition of Caseinolytic peptidase B in Bacterial Pathogens

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Caseinolytic peptidase B (ClpB) is a molecular chaperone involved in protein disaggregation and reactivation of highly aggregated proteins in bacterial pathogens. ClpB belongs to the Hsp100 subfamily of the AAA+ superfamily of ATPases (ATPases Associated with various Activities) which is associated with conformational remodeling of macromolecules. Similar to other Hsp100 proteins, ClpB is able to form ring-shaped hexamic oligomers and unfolds its substrate by threading it through a central pore in an ATP-dependent manner. ClpB has nucleotide binding sites at the interface between subunits which provide energy for unfolding of polypeptides. ClpB forms stable complexes with its substrates with assistance from other chaperones DnaK, DnaJ and GrpE. These proteins work in cooperation with ClpB and are required for the reactivation of aggregated proteins in the process of maintaining cellular proteostasis under severe heat stress conditions. ClpB is a promising target for the development of novel antimicrobials due to its essential role in the survival of bacterial pathogens and a lack of human orthologs. In a recent study, researchers found that N2, - N4 - dibenzylquinazoline - 2,4-diamine (DBeQ), a known p97 inhibitor, specifically inhibits the function of ClpB in *Escherichia Coli*. This review investigates the role of ClpB in the survival of bacterial pathogens during infection and its potential as a target for novel antimicrobial drugs.

This MAPS Team project is supported by the Center for Biomolecular Modeling and the Medical Professions Academy of Olathe North High School under the mentorship of Zachary Spaulding and Dr. Michael Zolkiewski, both of Kansas State University, Manhattan, KS.

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Abstract 2261

Real-time Antimicrobial Susceptibility and Metabolic Characterizations of an Environmental Strain *Stenotrophomonas maltophilia* NEB515

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Philips Akinwole

An increase in the nosocomial infections of *Stenotrophomonas maltophilia*; an emerging multidrug-resistant opportunistic human pathogen, has been projected. This expected increase necessitates the need to vigilantly monitor drug resistance in *S. maltophilia*. However, *S. maltophilia* is also used as a biocontrol agent for crops in sustainable agriculture and bioremediation strategies. Currently, it is challenging to distinguish beneficial (environmental) from harmful (clinical) *S. maltophilia* strains. Thus, continuous and real-time monitoring for reliable growth-dependent evaluation of antibiotic susceptibility of *S. maltophilia* may result in the discovery of effective treatments for infections and the identification of differentiating features among the isolates. Here, we used a real-time kinetic assay for simultaneous cultivation and time-resolved growth analysis of an environmental strain *S. maltophilia* NEB515 in a GN4F 96-well antibiotic test plate instead of end-point susceptibility testing. In addition, we determined the metabolic profile of *S. maltophilia* using the BIOLOGTM EcoPlates assay. A total of 24 antibiotics were tested at various concentrations in a 48 h experiment. Environmental *S. maltophilia* isolate was sensitive to 83% of the antibiotics used, including Amikacin, Piperacililin/Tazobactam constant 4, Ciprofloxacin, and Trimethoprim/Sulfamethoxazole. However, in the presence of antibiotics Ceftazidime, Ampicillin and Ampicillin/Sulbactan 2:1 ratio, it grew at higher rates compared to the control cultures (without antibiotics). A complex concentration-dependent effect was observed for Ticarcilin/Clavulanic acid constant 2 (TIM2). Growth of *S. maltophilia* was significantly reduced compared to control cultures up to 25 h in the presence of TIM 2 (16/2, 32/2, and 64/2) after which pronounced growth matched control cultures suggesting that growth stimulation may occur with extended exposure to sublethal antibiotic concentrations. The physiology profile results obtained from the EcoPlates after 120 h incubation showed that *S. maltophilia* metabolized an average of 58% of the 31 carbon sources to a varying extent. Growth dynamics of *S. maltophilia* in the EcoPlate was inhibited in the presence of i-Erythritolin (carbohydrate), L-Phenylalanine and L-Arginine (amino acids), α-Cyclodextrin (polymer) and seven of the carboxylic acids' substrates except α-Ketobutyric acid. Thus, the characterization of metabolic profiles of *S. maltophilia* in this study might have a promising role in the discovery of new targets for antimicrobial compounds. Our results support that though environmental strains such as *S. maltophilia* NEB515 present high susceptibility to several antimicrobials, there are concerns about the risk it may have for immunocompromised individuals, despite its confirmed biotechnological potentials and applications.

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ASBMB

Abstract 2297

Heterologous expression of *Pseudomonas aeruginosa* ATP synthase in *E. coli* to facilitate antibiotic discovery

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Ryan Steed

Bacterial multidrug resistance (MDR) is a prevalent and increasing threat, necessitating the constant development of new antibiotics, preferably with novel mechanisms of action to avoid existing resistance. Targeting the bioenergetic pathways of bacteria has shown promise in overcoming drug-resistance, as demonstrated by the diarylquinoline bedaquiline, which is effective against the ATP synthase of *Mycobacterium tuberculosis*. Expanding on this strategy, we are screening drug candidates to identify effective inhibitors of the ATP synthase of *Pseudomonas aeruginosa* (PA), an opportunistic, Gram-negative pathogen that already exhibits MDR in the clinic. To facilitate the design and testing of new inhibitors, we constructed a plasmid to express PA F1Fo ATP synthase in *Escherichia coli*. The plasmid, pASH20, derived from the ampicillin-resistant vector pBR322, contains the PA ATP synthase (atp) operon under the control of the native *E. coli* atp (unc) promoter and encodes an affinity tag on the N-terminus of the beta subunit to facilitate future F1Fo purification. Expression of functional ATP synthase was verified by growth of transformant *E. coli* on succinate minimal medium. Additionally, inverted membrane vesicles prepared from transformant *E. coli* showed *in vitro* ATP synthesis and hydrolysis activities. Successful expression of PA ATP synthase from pASH20 enables future mutagenesis and purification experiments to aid in the design of effective antibiotics targeting PA bioenergetics.

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Abstract 2299

Identifying Iron-Oxidizing Acidophiles in Acid Mine Drainage Sites in Southwest Colorado

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The High Country of Southwest Colorado is littered with hundreds of inactive and abandoned mining sites. Abandoned mines fill with water and leak out acidic water with high dissolved-metal concentrations, known as acidic mine drainage (AMD). Heavy metal contaminated water poses a significant threat to public health and can alter microbial compositions impacting plant, animal, and human health. Extremely acidophilic bacteria are widespread in AMD systems, but the metabolic potential, ecological functions, and evolutionary history of microorganisms is not well studied. These local mining sites provide an opportunity to study unique iron-oxidizing microorganisms. The current study combines experimental and whole genome sequencing approaches to investigate these iron-oxidizing, extreme acidophilic bacteria. Previously, 16S rRNA amplicon sequencing identified acidophilic microorganisms: Gallionella spp., "Ferrovum" spp., and Acidithiobacillus spp., and Leptospirillum spp; in sediment samples. We cultured bacterial colonies on iron-salts-purified (ISP) agar from acid mine drainage sediment and water samples. DNA extracted from one colony was amplified using the general 16S rRNA gene. A DNA library was prepared using the Rapid Sequencing Kit from Oxford Nanopore Technologies (ONT). The sample was sequenced using the ONT MinION sequencer. The preliminary data identified over 22 K high quality reads with an average of 1 K bases in length. Using EPI2ME What's In My Pot (WIMP) bioinformatics ONT tool, the sequences closely matched to an Acidiphilium spp. Poor DNA quality was observed and modification of media was optimized. Bacterial cultures were grown from glycerol stocks in a modified iron-salts-purified (ISP) liquid media. These cells were washed with ethylenediaminetetraacetic acid (EDTA) to remove metals and increase DNA quality for sequencing. Whole genome sequencing was performed on these bacteria using the ONT MinION sequencer. The genomes of these acidophiles contain numerous functional genes for iron and sulfur metabolism, nitrogen fixation and multidrug and heavy metal resistance. The goal of this project is to provide an approach for functional annotation of acidophile genomes to enable application to bioremediation methods and antibiotic resistance.

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Abstract 2306

Two dynamic, N-terminal regions are required for function in Ribosomal RNA Adenine Dimethylase family members

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Alex Collins, Jack Dunkle

Erythromycin resistance methyltransferase (Erm) dimethylates A2058 of 23S rRNA to m62A2058 using methyl group donor S-adenosylmethionine (SAM), resulting in a multidrug-resistant phenotype in bacteria posing a significant threat to public health. Key residues involved in SAM binding and rRNA binding have been thoroughly investigated. However, through the use of random mutagenesis, new areas of interest have been highlighted in the N-Terminal: a basic patch and a highly conserved area known as Motif X, both of which when mutated were associated with an erythromycin sensitive phenotype in ErmE. To further investigate this new discovery, we designed site-directed mutants of ErmE. The resulting mutants were characterized by *in vivo* phenotypic assays and *in vitro* assays including single turnover kinetics and RNA-affinity binding assays. These results suggest that the basic patch and Motif X of the N-terminal contribute to rRNA methylation in ErmE, and we are continuing to investigate if these areas are also important across the ribosomal rRNA adenine dimethylase (RRAD) family.

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Abstract 2312

Three critical regions of the erythromycin resistance methyltransferase, ErmE, are required for function supporting a model for the interaction of Erm family enzymes with substrate rRNA

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6-Methyladenosine modification of DNA and RNA is widespread throughout the three domains of life and often accomplished by a Rossmann-fold methyltransferase domain which contains conserved sequence elements directing S-adenosylmethionine cofactor binding and placement of the target adenosine residue into the active site. Elaborations to the conserved Rossman-fold and appended domains direct methylation to diverse DNA and RNA sequences and structures. Recently, the first atomic-resolution structure of a ribosomal RNA adenine dimethylase (RRAD) family member bound to rRNA was solved, TFB1M bound to helix 45 of 12S rRNA. Since erythromycin resistance methyltransferases are also members of the RRAD family, and understanding how these enzymes recognize rRNA could be used to combat their role in antibiotic resistance, we constructed a model of ErmE bound to a 23S rRNA fragment based on the TFB1M–rRNA structure. We designed site-directed mutants of ErmE based on this model and assayed the mutants by *in vivo* phenotypic assays and *in vitro* assays with purified protein. Our results and additional bioinformatic analyses suggest our structural model captures key ErmE–rRNA interactions and indicate three regions of Erm proteins play a critical role in methylation: the target adenosine binding pocket, the basic ridge, and the α4-cleft.

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Abstract 2322

Kinetics and mechanisms of osmotic regulation after osmotic and temperature shock in the parasite model *Crithidia fasciculata*

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Parasites experience a wide range of osmotic and temperature stresses during their complex life-cycles. We seek to characterize the kinetics of the molecular responses to concurrent osmotic and temperature shock in the model flagellated parasite *Crithidia fasciculata*. Most organisms undergo regulatory volume decrease (RVD) in response to hypotonic stress through release of small molecules including amino acids. A contractile vacuole (CV) unique to certain free-living and parasitic protozoa expels excess water from the cell. Consistent with historical literature, we found that faster CV cycling occurs at higher temperatures in the ciliate *Vorticella convallaria*, with similar activation energies across protozoan groups. *Crithidia fasciculata* cells are much more sensitive to hypotonic lysis under concurrent warm temperature shock (over the normal growth temperature of 27°C), perhaps due to overwhelming the CV cycling mechanisms. Real time light scatter assays of RVD kinetics in *Crithidia fasciculata* showed that RVD began within the first 90 seconds after exposure to 75% hypotonic shock, reducing volume to a rounded steady state compared to cells in isotonic buffer. RVD was slightly faster at warmer temperatures, and occurred across a wide temperature range (5–37°C). All cells recovered their normal volumes when returned to isotonic buffer after 5 min of hypotonic shock regardless of temperature. The CV H+-ATPase inhibitor Bafilomycin B1 inhibited RVD leading to *Crithidia fasciculata* cell swelling, but did not inhibit recovery in isotonic buffer. A ninhydrin assay for small amines (amino acids) showed a rapid release of amines from the parasites dependent on the level of hypotonic shock. Further studies are planned to clarify the complementary roles of CV cycling and small amine release in RVD kinetics at different temperatures. Understanding the mechanisms of CV cycling supports the development of anti-parasitic drugs targeting the CV.

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Abstract 2323

Mutagenizing the bacterial small protein AcrZ and observing its effects on antibiotic resistance in *E. coli*

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Amira Reyad, Danielle Reed, Carl Soderstrom, Owusua Ennin, Mona Orr

Bacteria antibiotic resistance is a pressing public health issue. One clinically relevant mechanism that leads to antibiotic resistance is through overexpression or mutations in efflux pumps that remove antibiotics from the cytosol. One of the most studied is the AcrAB-TolC complex from *Escherichia coli*. AcrB is the pump component and is a member of the highly conserved resistance nodulation division (RND) family. Past studies show that AcrZ, a 49 amino acid small protein, binds to AcrB and affects antibiotic resistance. However, the amino acids important for binding and resistance and the exact mechanism by which AcrZ affects AcrB remains unclear. The objective of this study is to discover the amino acids in AcrZ that are critical for AcrB pump function and thus affect antibiotic resistance. To determine this, we have mutagenized a 10 amino acid stretch of AcrZ with random hydrophobic amino acids and assayed the ability of these AcrZ variants to confer complete resistance. We used a bacterial split adenylate cyclase assay to screen the mutants that retain binding activity, subcloned them to an expression vector to assay antibiotic activity, and sequenced them to record which hydrophobic amino acids were conserved. The acrZ mutants are tested for antibiotic resistance by antibiotic gradient plate assays and minimum inhibitory concentration (MIC) assays of various antibiotics. Currently, results do not identify any conserved amino acids or conserved motifs in the amino acid sequence important for retaining binding activity or antibiotic efflux activity. However, we have identified an AcrZ variant that appears to increase drug resistance above the wild type sequence and an AcrZ variant that decreases drug resistance to below the deletion of acrZ. These variants will be subjected to further structure-function studies to determine how AcrZ mediates AcrB activity.

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Abstract 2328

Characterization of rmdA and rmdB in *Streptomyces scabies*

Ashni Patel, *Otterbein University*

Jennifer Bennett

Streptomyces scabies is a gram-positive bacterium that causes a scab disease in potatoes. The bacterium itself is filamentous and produces spores which allows it to spread and reproduce. The rmdA and rmdB genes in *S. scabies* are used in the cyclic-di-GMP signaling pathway. This pathway is responsible for regulating many processes including cell cycle progression, differentiation, and antibiotic production. RmdA and B are phosphodiesterases that break down the second messenger, cyclic-di-GMP. The specific question we want to address is what role rmdA and rmdB play in the development of *S. scabies* and its ability to cause disease in potatoes. Our research would allow us to study the pathogenicity of *S. scabies* which is on the prevalent pest list, and it will also potentially enable us to manipulate antibiotic production on an industrial level. Our lab has already constructed a rmdB mutant and will continue with a deletion of the rmdA gene, finishing with a rmdA and rmdB double mutant. We are constructing the rmdA deletion using the lambda Red recombinase system (REDIRECT). REDIRECT works by first designing a forward primer sequence that will provide a region of homology for 39 bases that includes the start codon and flanking DNA directly upstream of the gene. A reverse primer was then designed to provide homology to the stop codon and downstream sequence of rmdA. Both primers also contain 19–20 bases to amplify an apramycin resistance gene cassette that will ultimately replace the rmdA gene. We have transformed the lambda Red recombinase containing plasmid and the apramycin resistance cassette that has flanking regions of homology to the gene to be deleted into *Escherichia coli* to create the deletion. The new cosmid will then be introduced into the mating strain of *E. coli*. Then we conjugate that accordingly into *Streptomyces scabies* to obtain the deletion mutant. The next steps would be to examine the phenotypes of the deletion mutant candidates and compare them to the wild-type strain and the defects in the rmdB mutant that we have already constructed. This can be done by streaking the strains onto plates and introducing the strains onto potatoes and radish seedlings to observe potential changes to pathogenicity. We are currently also in the process of using Real-Time PCR to follow gene expression of virulence factors as well as extending our bioinformatics analyses.

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Abstract 2369

Discovering and assessing novel epitopes of *Bordetella pertussis* to improve the whooping cough vaccine through immunopeptidome analysis and immune recognition assays

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Mohamed Shamseldin, Eva Verzani, Karl Clauser, Jesse Hall, Steven Carr, Jennifer Abelin, Purnima Dubey

Cases of whooping cough, a respiratory disease caused by the gram-negative bacterial pathogen *Bordetella pertussis*, is increasing despite high global vaccination rates. Whooping cough is highly contagious and mimics the symptoms of a common cold, but can quickly become life-threatening to infants. The resurgence of pertussis may be associated with the transition from a whole-cell pertussis vaccine (wPV) to an acellular vaccine (aPV) made up of 1–5 components of *B. pertussis*. aPV-component selection pressure may have contributed to antigen-loss strains currently circulating. In addition, current pertussis vaccines are limited by being designed to activate antibody responses and not a T cell response, contributing to short-lived aPV-generated immunity. Therefore, whooping cough vaccines can be improved by identifying and incorporating peptides that elicit a CD4+ T cell response, which is critical for long-term immunity against both infections and transmission. To address this challenge, we propose directly analyzing *B. pertussis* proteins that are processed and presented by the major histocompatibility complex II (MHC-II) on dendritic cells exposed to heat-killed *B. pertussis* using mass spectrometry-based immunopeptidomics. Immunopeptidome analysis revealed a cache of novel MHC-II-presented peptides derived from *B. pertussis* that are candidates for integration into a more fortified aPV. To further investigate these putative antigens, we assessed their predicted MHC-affinity, relative abundance, conservation across circulating strains, and *ex vivo* recognition by wPV-immunized mice and human CD4+ T cells. Future validation efforts will include *in vivo* immune recognition analysis and pertussis infection prevention assays. Through leveraging the power of immunopetidomics, we uncovered novel antigens presented by MHC-II that are recognized by the immune system of wPV-immunized mice and humans and elicit a CD4+ T cell response. These new epitopes are promising candidates for improving aPV and preventing the growing number of whooping cough cases.

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Abstract 2404

Unexpected heme metabolism by GI tract and environmental anaerobes

Jennifer DuBois, *Montana State University*

Research on microbial iron metabolism has historically focused on the host-pathogen relationship, where the host suppresses pathogen growth by cutting off access to iron. Less is known about how host iron is shared with bacterial species that live commensally in the anaerobic human GI tract, typified by members of phylum Bacteroidetes. While many facultative pathogens avidly produce and consume heme-iron, most GI tract anaerobes are heme auxotrophs whose metabolic preferences we aimed to describe. Understanding iron metabolism by model microbiome species like *Bacteroides thetaiotaomicron* is essential for modeling the ecology of the GI tract, which serves the long-term biomedical goals of manipulating the microbiome to facilitate host metabolism of iron and remediate dysbiosis and associated pathologies (inflammation, cancer). This talk describes recent work identifying heme metabolic capabilities in Bacteroides species with unexpected parallels in terrestrial methanogenic Archaea.

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Abstract 2423

Ribose as a signal to emerge from dormant state and lower RpoS levels

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Julia Lockart, Celeste Peterson

RpoS activates the dormant response of *Escherichia coli* under harsh environmental conditions. In this state of hibernation, the bacteria can survive prolonged starvation and are more resistant to antibiotics. Currently, more is known about signals that activate the dormancy state rather than the signals involved in the pathway of coming out of this state. A screen found that the ribose metabolism gene rbsD lowered RpoS levels when overexpressed and slightly increased RpoS levels when knocked out. Using a series of different LacZ fusions to RpoS, we found that rbsD overexpression lowers RpoS translation but only has a minimal effect on transcription and protein stability. It was determined that in order for rbsD to have an effect on RpoS, the sRNA chaperone hfq is crucial. In minimal media with ribose, the levels of RpoS decreased compared to minimal media with glyercol. The effect of ribose in lowering RpoS levels was abolished when the rbsD gene was knocked out. In conclusion, ribose is a key signal in lowering RpoS levels and therefore inducing the bacteria to exit the dormant state under favorable conditions.

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Abstract 2464

Dysbiosis of Gut Microbiota Increases Colon Cancer Risks in a Novel Mouse Model of Colitis

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Siyuan Liang, Guanying Xu, Erick Vazquez, Yuan-Xiang Pan, Hong Chen

The etiology of IBD remains unclear, however, the interactions between excessive immune response from host and environment are predicted to be the primary cause of this disease. Additionally, gut microbiota has been well-known as one factor affecting IBD's pathophysiology. The novel hnRNPI knockout (KO) in intestinal epithelial cells activates the NF-κB pathway and causes spontaneous colitis. However, whether gut microbiota changes in KO mice are the cause or consequence of developing spontaneous colitis remains unclear. We hypothesize that hnRNPI gene knockout in intestinal epithelial cells contributes to gut microbiota dysbiosis and increases the risk of developing colon cancer. Six-week-old KO and WT mice were challenged using DSS for a week. Fecal samples at the baseline (day 0) and final day of DSS challenge (day 7) were collected. Body weight was monitored, and the Disease Activity Index (DAI) was measured. Organ samples, including liver, spleen, and colon, were measured during the necropsy. DNA from fecal samples was isolated and analyzed by sequencing V3-V4 region of 16S rRNA gene. *Paraclostridium bifermentans* and *Enterococcus faecalis* were highly abundant in KO mice, while *Dubosiella newyorkensis* was reduced in the KO mice. KO mice showed higher DAI than WT mice after DSS challenge. Alpha and beta diversity were significantly different in KO mice after DSS compared to baseline, while WT mice showed no significant difference in alpha diversity in baseline and after DSS challenge. Specifically, *P. bifermentans*, *Clostridium paraputrificium*, and *Lactococcus garvieae* were highly abundant in KO DSS mice, while *D. newyorkensis* and Malacoplasma muris remained reduced in KO mice. Metagenomic analysis of 16S rRNA genes showed that gut microbiota changes in hnRNPI KO mice are highly associated with cancer pathways after DSS challenge. Our study firstly demonstrated that *P. bifermentans*, *E. faecalis*, and *D. newyorkensis* are potential markers of gut microbiota dysbiosis in the hnRNPI KO mice. During the DSS challenge, KO mice are more susceptible to developing colitis. Interestingly, gut microbiota shift in KO mice affects the increased risk of colon cancer. This research agrees with the current literature regarding the increased risk of colorectal cancer (CRC) incidence in ulcerative colitis. Further research will investigate the crosstalk between gut microbiota and the immune system during colitis, deepening our understanding of dysbiosis in microbiota and colon cancer risk.

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Abstract 2473

Isolation and characterization of bacteriophages infecting metal iron-reducing bacterium *Shewanella oneidensis* MR-1

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Vivek Mutalik, Roniya Thapa Magar

Bacterial viruses, or bacteriophages (phages) represent the most abundant biological entities on earth. Phages are known to attack specific bacteria, but their specificity of interaction is deeply under-characterized and focused only on model bacterial systems. The goal of this project was to isolate and characterize double-stranded DNA phages for *Shewanella oneidensis* MR-1, a bacteria with applications in electro-biotechnology. Toward this goal, it was enriched with more than 35 environmental samples with *Shewanella oneidensis* MR-1 and carried out plaque (a zone of clearance) assays to isolate 10 different phages. Then serial dilutions and spot assays were performed to estimate phage numbers in plaque forming units and extracted their genomic DNA by the Promega Wizard method. The phage samples are being prepared for image analysis by Transmission Electron Microscope (TEM) and genome sequencing using Illumina genome sequencing. Finally, assays are being planned to identify phage-specific host receptors using barcoded loss-of-function mutant libraries. By studying *S. oneidensis* MR-1 interaction with diverse phages, we will gain insight into the host genetic factors that render bacteria susceptible or resistant to killing by specific phages.

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ASBMB

Abstract 2480

Characterization of Novel Developmental Genes in a Pharmacologically Important Bacterium

Jennifer Bennett, *Otterbein University*

Streptomyces species live in soils worldwide and produce over two-thirds of the commercially important antibiotics as well as a variety of other secondary metabolites. With a large 8.7 megabase genome, encoding over 7000 genes, the majority of these genes and corresponding products are largely uncharacterized. In this study, an *in vivo* transposon system was used to randomly mutagenize wild-type *Streptomyces coelicolor* and isolate hundreds of mutants that show distinct colony phenotypes. The transposon insertion sites for 35 of these mutants were identified by isolating chromosomal DNA and subjecting the DNA to restriction enzyme digestion, followed by ligation of these fragments into small circular pieces of DNA. Then Inverse PCR, using primers that anneal to the known transposon sequence, was employed to obtain a linear PCR product for each mutant. A nested primer that also anneals to the known transposon sequence was used in Sanger Sequencing to identify the unknown region and determine the precise transposon insertion site using BLAST searches. Upon sequence analysis it was found that only a small number of mutants contained insertions in well-studied developmental genes, including cell division gene ftsI and sporulation genes whiE and whiH. Uncharacterized genes and genes with very little published research were discovered in mutants displaying a strong chromosome segregation defect, sporulation defects, delays in development and/or changes in pigmented antibiotic production. These mutants have been characterized using macroscopic phenotyping on various media, phase-contrast and fluorescence microscopy, bioinformatics, and various other methods. As the functions for many of these uncharacterized genes are uncovered, more knowledge about bacterial development and antibiotic production can be harnessed for medicine and industry. New cell division genes represent potential antibiotic targets in pathogenic species, while new developmental and regulatory genes may provide ways to manipulate antibiotic production that can be used in medicine and agriculture.

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Abstract 2518

Omics level science to connect the nitrogen and carbon cycles

Lisa Stein, *University of Alberta*

Technologies to reduce GHG emissions must take microorganisms into account as this invisible majority is largely responsible for production and consumption of methane and nitrous oxide, the second and third most important GHGs in causing global warming. Methanogenesis produces most of the methane emitted to the atmosphere, whereas methanotrophic microbes account for methane consumption prior to its emission, and ca. 1% of atmospheric methane consumption. The primary source of nitrous oxide to the atmosphere is from nitrifying and denitrifying microorganisms whose activity has accelerated over the past 60 years due to anthropogenic input of reactive-N to the biosphere. Interestingly, methanotrophic and nitrifying microbes share common enzymes and metabolic pathways, enabling both groups to produce or consume GHGs depending mainly on redox potential and nitrogen availability. Using the bacterium *Methylomicrobium denitrificans* FJG1 as a model system, we collected RNAseq, proteomics and metabolomics data across 6-point growth curves to examine the dynamics of gene expression as a function of oxygen and nitrogen availability. A gene regulatory network of the RNAseq data showed different topologies with either ammonium or nitrate as the N-source, as nitrate is required to induce methane-dependent denitrification. With a genome-scale metabolic model under construction, the omics data point to a division of labor in *M. denitrificans* between fermentation and denitrification at the onset of anoxia. Extrapolating to natural ecosystems, a similar division of labor has been observed in anoxic freshwater lakes wherein methanotrophs use alternative electron acceptors to consume methane while providing organic molecules from fermentation to cross-feed other microbial populations. However, when nitrate is the alternative electron acceptor, nitrous oxide is produced proportionally to methane consumption. This case-study demonstrates the need to consider interconnectedness and coevolution of microbial functionality, and to apply omics-based systems biology models when developing and implementing GHG reduction strategies.

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Abstract 2590

Gut microbial dysbiosis and host fatty acid metabolites in inflammatory bowel disease

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Acylcarnitines are membrane permeable intermediary metabolites in fatty acid beta-oxidation. Plasma levels of these metabolites are elevated in individuals with dysfunctional mutations in this pathway and in response to exercise and fasting. Our group has previously shown that bile derived acylcarnitines can be utilized by healthy colonic epithelium as an energy source when short chain fatty acids are limited and that their levels are elevated in the feces of patients with inflammatory bowel disease (IBD). In a new analysis of data collected from a pediatric IBD cohort, we identified a strong positive correlation between fecal acylcarnitine levels, Enterobacteriaceae and the bacterial cai operon needed for carnitine consumption. Based on data from human subject, mouse, and bacterial culture studies, we now have evidence that the gut microbiota can consume these host-derived metabolites in taxon specific ways. Carnitine can serve as an alternative electron acceptor to enhance *Escherichia coli* utilization of glycerol under anaerobic conditions. This function may help explain the relationship between diet and IBD where previous studies have reported that red meat consumption is both epidemiologically associated with the prevalence of IBD and exacerbates colitis in animal models. Further investigation into the relationship between elevated luminal acylcarnitines and their effect on the gut microbiota may provide novel insights into IBD progression.

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Abstract 2598

Centella asiatica extract inhibits *Mycobacterium tuberculosis* growth in rat tuberculosis models

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Background and Aims: The covid-19 pandemic has reversed years of progress in the fight to end tuberculosis. So, the discovery of new drugs as antituberculosis is very much needed. Our previous studies have shown that the extract of *Centella asiatica* is able to inhibit the growth of *Mycobacterium tuberculosis in vitro* and requires further research. The aims of this study is to prove the effect of *Centella asiatica* inhibit *Mycobacterium tuberculosis* in rat model tuberculosis.

Methods: The protocol in this study was approved by the veterinary ethics committee of Airlangga University. The rat tuberculosis model was induced by intrathecal injection of a suspension of *Mycobacterium tuberculosis* strain H37 Rv. Twenty-eight tuberculosis rat were randomly divided into four groups. Groups 1,2, and 3 were treated with ethanol extract of *Centella asiatica* at 375 mg/kgBW, 750 mg/kgBW and 1500 mg/kgBW, and the fourth group was the control group. *Centella asiatica* extract is administered orally via an intragastric feeding tube for two weeks, once daily At the end of the experimental period, rats were sacrificed by cervical decapitation. The left lung tissue was taken aseptically and cultured on Middlebrook 7H10.

Results: The results showed that there was no bacterial growth on the culture media in the group that received *Centella asiatica* extract at a dose of 750 and 1500 mg/kg BW.

Conclusion: The conclusion in this study, that *Centella asiatica* extract inhibit the growth of *Mycobacterium tuberculosis* at doses of 750 and 1500 mg/kg BW.

We thank the Directorate of Research and Community Services, the Directorate General of Higher Education, and Ministry of Education and Culture in Indonesia for the financial support

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ASBMB

Abstract 2600

Role of *Rhodospirillum rubrum* UbiT, UbiU, and UbiV proteins in anaerobic rhodoquinone biosynthesis

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Rhodoquinone (RQ) is an electron carrier that participates in the anaerobic metabolic pathways of some bacteria and eukaryotic organisms. Due to its significance in cellular metabolism, understanding the biosynthesis of RQ may precede the development of novel anti-parasitic and anti-microbial drugs. Ubiquinone (UQ), a structurally similar electron carrier used in aerobic respiration, is a precursor for the biosynthesis of RQ in some bacteria and protists that have the rquA gene. While RQ is not produced by *Escherichia coli*, UQ is synthesized via two separate biological pathways depending on oxygen availability. The anaerobic pathway for UQ biosynthesis in *E. coli* relies on UbiT, UbiU, and UbiV proteins for hydroxylation, while the aerobic pathway utilizes a Ubi complex with UbiI, UbiH and UbiF monooxygenase proteins. *Rhodospirillum rubrum* produces RQ from UQ, and in addition to the rquA gene, its genome contains homologs of most of the ubi genes. To further study the anaerobic biosynthesis of RQ, a mutant strain of *R. rubrum* was produced by deletion of the ubiT-ubiU-ubiV operon. A vector was designed for gene knockout using the pUX19 suicide plasmid constructed with the 5' and 3' flanking regions of the genes and a gentamicin resistance cassette. Conjugation of *R. rubrum* with S17-1 *E. coli* containing the knock-out vector, homologous recombination, and antibiotic selection allowed for isolation of the mutant. Growth studies will be performed to determine the dependence of RQ biosynthesis on UbiT, UbiU, and UbiV under both aerobic and anaerobic conditions.

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Abstract 2613

A family of spore lipoproteins stabilize the germination apparatus through altering membrane fluidity in *Bacillus subtilis* spores

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To return to a vegetative state, dormant bacterial spores must undergo the process of germination, which can be divided into two stages. In most species, stage one is triggered by sensing of nutrient germinants and is characterized by the release of calcium-dipicolinic acid (DPA) and various cations, while stage two is characterized by spore cortex degradation and full rehydration of the spore core. All these steps are mediated by membrane-associated proteins, including germinant receptors, the major DPA channel, and a key cortex lytic enzyme, SleB, and all these proteins have exposure on the outer surface of the membrane, a hydrated environment where they are potentially subject to damage during dormancy. A family of highly abundant spore lipoproteins, including YlaJ, which is expressed from the sleB operon in some species, are present in all Bacillus and Clostridium species that express SleB. *B. subtilis* possesses four proteins in this family, and prior studies have demonstrated that two of these are required for the most efficient spore germination. Genetic studies of strains lacking all combinations of these four genes now reveal that all four play roles in ensuring efficient germination, and that they affect multiple steps in this process, including germinant sensing, DPA release, and SleB stability. Electron microscopy does not reveal significant changes in spore morphology in strains lacking lipoproteins. Generalized polarization measurements of a membrane dye probe indicate that the lipoproteins decrease spore membrane fluidity. These data suggest a model in which the lipoproteins form a macromolecular structure on the outer surface of the inner spore membrane, where they act to stabilize the membrane and potentially interact with other germination proteins, and thus stabilize the function of multiple components of the germination machinery.

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Abstract 2616

Elucidating the iron acquisition mechanisms that enable *Staphylococcus aureus* growth within macrophages

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Ronald Flannagan, David Heinrichs

The macrophage phagolysosome represents a nutrient deplete antimicrobial niche. Despite this, *Staphylococcus aureus* can replicate within the mature phagolysosome which necessitates that *S. aureus* acquire nutrients, such as iron, for growth. To understand which iron acquisition loci *S. aureus* strain USA300 uses to replicate within macrophages a mutant, Δ4, was created that lack the sfa, sbn, cntKLM and feoAB loci encoding staphyloferrin A/B, staphylopine, and the ferrous iron transporter FeoAB, respectively. Growth analysis under iron restricted conditions used using mock macrophage infection assays revealed that Δ4 cannot grow and in fact loses viability. In contrast upon macrophage infection and gentamicin protection, Δ4 can replicate indicating the bacteria derive iron from within the macrophage. Fluorescence based bacterial proliferation assays in conjunction with confocal microscopy confirmed that Δ4 commences growing intracellularly akin to wild type *S. aureus*. Using Δ4 we sequentially inactivated the sst and fhuCBG loci that encode catechol and hydroxamate siderophore utilization giving rise to strains Δ5 and Δ6, respectively. Remarkably, these strains, despite failing to grow *in vitro* under conditions of iron restriction in the presence of norepinephrine or deferoxamine, retain the ability to proliferate upon macrophage infection. Interestingly, mutagenesis of hemB also did not attenuate growth of *S. aureus* in macrophages indicating the bacterial must acquire heme, however this occurs through an isdEF-independent mechanism. Together, these data indicate that *S. aureus* is not reliant on known siderophore iron acquisition systems for growth within macrophages and ongoing studies are aimed at identifying the systems that contribute to intracellular growth.

Canadian Institutes of Health Research.

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Abstract 2621

Trypanosoma cruzi dysregulates piRNAs targeting IL-6 signaling pathway in primary human cardiac fibroblasts during acute infection

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Trypanosoma cruzi, the causative agent of Chagas Disease, causes severe morbidity, mortality, and economic burden worldwide. Due to globalization, the parasite is now present in most industrialized countries. About 30–40% of chronically infected individuals will develop cardiovascular, neurological, and/or gastrointestinal pathologies. Cardiomyopathies induced by chronic parasite infection include organ enlargement and fibrosis, accompanied by significant alterations in the cellular expression of profibrotic inflammatory molecules. Accumulating evidence suggests that the parasite induces alterations in host gene expression profiles to facilitate infection and pathogenesis. The role of regulatory gene expression machinery during *T. cruzi* infection has yet to be elucidated. In this study, we aim to examine the role of piwi-interacting RNAs (piRNAs), a newly defined class of small noncoding RNA molecules, during the early phase of *T. cruzi* infection in primary human cardiac fibroblasts (PHCF). Recently published studies suggest that IL-6/STAT3 signaling plays an important role cardiac fibrogenesis in Chagasic murine models. Our previous bioinformatic analysis of parasite challenged PHCF identified significant modulation of piRNAs targeting IL6 signaling pathway components. To validate this, we performed RT-qPCR of IL6, SOCS3, and piRNAs predicted to target IL6 and SOCS3. We then evaluated protein expression for p-STAT3 (Y705), STAT3, and SOCS3 in cell lysates and IL-6 secretion in condition media of parasite challenged PHCF. We observed dysregulation of piRNAs, mRNA and protein expression of IL-6 signaling molecules. This data corroborates recent studies that suggest STAT3 plays a role in *T. cruzi* pathogenesis.

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Abstract 2630

The LexA-Like Repressor UmuDAb and It's Corepressor DdrR Affect Cell Division Phenotypes in *Acinetobacter baumannii*

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Acinetobacter baumannii is a multi-drug resistant pathogen commonly found in hospital-acquired infections. We are studying the effects of overexpression of UmuDAb and DdrR on this bacterium's cell length to help understand how it controls its response to antibiotic exposure and DNA damage, as well as how these exposures can halt its cell division. UmuDAb and DdrR corepress error-prone polymerases in *Acinetobacter baumannii* until after DNA damage. However, they also regulate and induce non-polymerase genes, prompting us to examine DdrR and UmuDAb mutants for DNA damage-related phenotypes. We observed that ddrR and umuDAb mutants possess two DNA damage-related cell division phenotypes: growth sensitivity and alterations in filamentation. The ddrR mutant's growth was affected more than wild-type (WT) cells after DNA damage. We measured (by OD600) and compared cell growth in the presence and absence of DNA damage (mitomycin C; MMC) in late log-early stationary phase (4–6 hours). WT cell growth after MMC treatment was ~85% of control growth, whereas a ddrR insertion mutant's growth after MMC treatment was only 70%. Cell filamentation, a common response to DNA damage, was observed in WT but not ddrR mutant cells (40+ cells for each strain measured with MicrobeJ and analyzed by ANOVA). Interestingly, umuDAb mutant cells were longer than WT cells even under control conditions. UmuDAb expression from a plasmid-based arabinose-inducible promoter, confirmed by western blotting, restored WT cell lengths. Furthermore, these longer umuDAb cells further increased in length after DNA damage, and this filamentation depended on UmuDAb self-cleavage and relief of repression. Our data show that both the amount of DdrR and UmuDAb affects cell lengths.

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Abstract 2647

Antibiotic resistance of *Salmonella enterica* serovar Enteritidis mediated by EnvZ/OmpR two-component system in response to β-lactams

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Sang Ho Choi

Outer membrane porins (OMPs) of Gram-negative bacteria including *Salmonella enterica* serovar Enteritidis allow the entry of small hydrophilic antibiotics. S. Enteritidis is a major food-borne pathogen, and infection by S. Enteritidis often results in severe invasive disease, which requires antibiotic treatments. Although modulation of OMP composition is frequently observed in multidrug-resistant bacteria, little is known about the mechanisms by which S. Enteritidis modulates its OMP composition to enhance antibiotic resistance. Here, we found that the S. Enteritidis strain with an active state of EnvZ/OmpR two-component system is highly resistant to multiple antibiotics, especially β-lactams. Transcriptome analysis showed that the active state of EnvZ/OmpR induces differential expression of several OMP genes, including SEN1522, SEN2875, ompD, and ompW. Interestingly, response regulator OmpR above a certain cellular level is required for regulation of the four OMP genes, and phosphorylation of OmpR is not necessary for repression of ompD and ompW. Phenotypic analysis revealed that ompD is a key OMP gene responsible for the EnvZ/OmpR-mediated antibiotic resistance in S. Enteritidis. It is noteworthy that EnvZ/OmpR represses the ompD expression by responding to the presence of β-lactams and provides S. Enteritidis with direct benefits for survival. Taken together, this study suggests that EnvZ/OmpR remodels the OMP composition of S. Enteritidis in response to β-lactams and enhances antibiotic resistance.

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Abstract 2663

Rising level of pulmonary dioxin might trigger CMV and HSV-1 reactivation via bHLH/PAS/AhR:ARNT transcription pathway in lungs of immunocompetent COVID-19 patients with ARDS

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Irina Shur

Study objective. It has been shown that human common viruses are new target genes for host cell dioxin receptor transcriptional (AhR-ARNT) complex initially proven to up-regulate mammalian genes containing dioxin-response elements (DRE) in the promoters [doi:10.1016/j.ijid.2012.05.265]. Initially, transactivation of HIV-1 and HBV by 2,3,7,8-tetrachlodibenzo-p-dioxin (TCDD) at low nanomolar range was demonstrated [doi:10.3109/00498259309057034]. Noteworthy, transactivation of human cytomegalovirus (CMV) was shown with 0.3 ppt dioxin, i.e. lower than its current background level in the general population (~3.0 ppt). Recently, reactivation of CMV infection was found to influence worse clinical outcome following SARS-CoV-2 infection (doi: 10.1186/s12979-020-00185-x). Other findings showed that CMV and herpes simplex virus 1 (HSV-1) reactivation were observed in immunocompetent patients with COVID-19 acute respiratory distress syndrome (ARDS) (doi.org/10.1186/s13054-020-03252-3). Addressing occurrence of Herpesviridae reactivation in severe COVID-19 patients, and still unspecified real triggers of CMV and HSV-1 reactivations, we tested TCDD, which current body burden (DBB) ranges from 20 pg/g (TEQ in fat) in general population to 100 pg/g in older people. Methods. In Silico quantitation of active DRE in promoters of viral genes. Virus DNA hybridization assay. Clinical and epidemiological analyses. Results and Discussion. In this study, a computational search for DRE in CMV and HSV-1 genes was performed by SITECON, a tool recognizing potentially active transcriptional factor binding sites. In silico analysis revealed in regulatory region of CMV IE genes from 5 to 10 DRE, and from 6 to 8 DRE in regulatory region of HSV-1 IE genes. We established that a low picomolar TCDD can trigger up-regulation of CMV and HSV-1 genes via AhR:Arnt transcription factor in macrophage(doi.org/10.1016/j.ijid.2012.05.265) and glial human cell lines (doi.org/10.1016/j.jalz.2016.06.1268), respectively. In fact, viral reactivation may be triggered in COVID-19 ARDS patients by higher pulmonary TCDD concentrations, because "lipid storm" within lungs of severe COVID-19 patients has been recently reported (doi.org/10.1101/2020.12.04.20242115). TCDD is known as the most potent xenobiotic, which bioaccumulates and has estimation half-life in humans of up to 10 yr. Due to hydrophobic character (Log P octanol/water: 7.05), TCDD partitions into inflammatory lipids in lung tissue thus augmenting its local concentration. Population-based epidemiological data on SARS-CoV-2 first wave of pandemic revealed high level of CMV seropositivity and cumulative mortality rate 4.5 times in Lombardi region of Italy, where after Seveso industrial accident TCDD plasma level in pre-exposed subjects is 15 times the level in rest of Italy (doi.org/10.3389/fpubh.2020.620416). Also, Arctic Native (AN) peoples consume dioxin-contaminated fat in seafood and have TCDD DBB, i.e. 7 times that in general population. To the point of this paper, their COVID-19 mortality is 2.2 times of that among non-AN Alaskans (doi: 10.15585/mmwr.mm6949a3). Conclusion: TCDD in the picomolar range may trigger CMV expression in lung cells and commit virus to the lytic cycle, which can be applied to reactivation of Herpesviridae infection in immunocompetent patients with COVID-19 ARDS syndrome.

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ASBMB

Abstract 2685

A potential role of protein phase separation in localization to a bacterial carbon-fixing organelle

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Bacterial Microcompartments (BMCs) are a widespread class of prokaryotic organelle that help regulate metabolism. Generally, BMCs utilize a semi-permeable protein shell to encapsulate specific sets of key enzymes and their substrates. The best studied example of a BMC is the carboxysome found in autotrophic bacteria which encapsulates the enzyme rubisco to help drive CO2 fixation. Using carboxysomes as a model system, we aim to understand how BMCs are spatially regulated to ensure their proper function and maintenance within a population. We recently identified the Maintenance of Carboxysome Distribution (Mcd) system, consisting of the two proteins McdA and McdB, which are both necessary for carboxysome spatial regulation. Our bioinformatics has shown that McdA and McdB homologs are found near hundreds of diverse BMC operons, but the mechanism of how these proteins interact with BMCs remains unclear. Here, using microscopy and biochemistry, I show that a conserved tryptophan is necessary for McdB to associate with carboxysomes in diverse autotrophic bacteria. Notably, mutating this tryptophan does not seem to affect McdB structure or oligomerization, but does affect the ability of McdB to phase separate *in vitro*. Intriguingly, substituting the tryptophan with other aromatic residues only slightly affects phase separation, and these substitutions have intermediate effects *in vivo*. Therefore, the aromaticity of this conserved residue can tune McdB phase separation *in vitro* and its association with carboxysomes *in vivo*, suggesting a potential link between the two. Lastly, I show that basic amino acids also contribute to McdB phase separation *in vitro*, suggesting a cation-pi network with the aromatic residues that stabilizes this process. The results here provide mechanistic insight into carboxysome spatial regulation as well as generally advance our understanding of BMCs and protein phase separation.

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Abstract 2706

AMR Enzymatic inactivation mechanism attributed to fosA Gene antibiotic resistance among Covid 19 hospitalized patients

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Introduction: The prevalence of antimicrobial resistance genes ARGs and their resistance genetic mechanisms among Covid-19 patients are yet to be identified. The human microbiome is a significant reservoir of antimicrobial resistance genes. The overprescription of antimicrobials can select multi-resistant bacteria and modify the repertoire of ARGs in the gut. The World Health Organization has reported 148 million hospitalized cases worldwide.

Objective: The purpose of the current study is to explore the genetic mechanisms of antimicrobial resistance among hospitalized COVID-19 patients, furthermore, to review their antibiotic resistance gene occurrence. Methodology 438 Microbiome of clinical hospitalized COVID-19 positive cases with 11 129 isolates were downloaded from the EMBL's European Bioinformatics Institute and the NCBI Pathogen Detection using the following keywords AMR, mechanism of resistance, and COVID-19 SARS CoV 2 bacterial Infection. We also have used the Comprehensive Antibiotic Resistance Database Card, and RESfinder are used for the metagenomics analysis based on programming languages JavaScript and R (v. 4) for data analysis.

Results: We explored the AMR diversity among prevalent microbes(n = 410), including *Klebsiella pneumoniae*, *Acinetobacter baumannii E. coli*, Salmonella, Enterobacter and *Pseudomonas aeruginosa*. We found that Enzyme activation (72.7%) was the most prevalent mechanism due to the fosA gene 54.5%. Then the aadA2 gene (18%) and catA1 gene(9%). Moreover, the Increased efflux mechanisms were detected in *Escherichia coli* towards Quinolone using the oqxA gene (17.3%). FosA was also intermittently found in Salmonella (9.8%), and *Pseudomonas aeruginosa* (7.8%).

Conclusions: We anticipate that FosA homologous is prevalent in Gram-negative bacterial infections among hospitalized COVID-19 patients, and it encodes for Fosfomycin resistance. The findings might shed light on controlling Fosfomycin resistance among hospitalized patients with COVID-19.

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Abstract 2707

Towards development of a CRISPRi system in vaginal Lactobacillus and genomic sequencing of vaginal microbiota

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The association of genes with phenotype is vital in gaining a more complete understanding of the vaginal microbiome and its role in human health, but most vaginal microbiota lack the genetic tools needed to do so. CRISPR interference (CRISPRi) is an approach to block gene expression using a catalytically inactive Cas9 protein (dCas9) and single guide RNAs. The central goal of this project was to examine the functions of bacterial genes and their role in host-microbe interactions through (1) the development of an effective CRISPRi system in vaginal Lactobacillus and (2) genomic sequencing of vaginal microbiota. To this end, we constructed a set of plasmids to optimize and perform CRISPRi. Using a Nanoluciferase reporter gene, we were able to confirm inducible gene expression in *Lactobacillus gasseri* using the PXyl TetO2 system. However, repression of the cpsE gene with CRISPRi was unsuccessful, demonstrating the need for further optimization. We also performed whole genome sequencing of vaginal swab isolates cultured on sheep blood agar using the Oxford Nanopore MinION platform. We obtained 18 closed genomes, many from non-Lactobacillus species. Future work will focus on validating the CRISPRi system in *L. gasseri* and applying it into additional vaginal Lactobacillus species.

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Abstract 2714

The role of histamine in iron acquisition by multidrug-resistant *Acinetobacter baumannii*

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Histamine is a potent immunomodulator that plays a key role in the innate immune response. Mast cells and basophils release histamine upon exposure to an antigen. The biological effects of histamine are diverse and include increasing vascular permeability and enhancing mucus production. Increased blood vessel permeability functions to allow escape of immune cells into the damaged tissue, allowing them to directly engage the antigen. Interestingly, the emerging opportunistic and extensively drug-resistant pathogen *Acinetobacter baumannii* also produces histamine. *A. baumannii* causes an array of infections, including bacteremia and pneumonia, where mortality rates can exceed 35%, but little is known about the factors that contribute to its pathogenesis. In *A. baumannii*, histamine appears to be produced as a key precursor molecule to production of the siderophore acinetobactin, an iron-binding molecule that facilitates bacterial capture of this essential nutrient. Here, using wild-type (WT) *A. baumannii* and a mutant impaired for the decarboxylation of histidine to histamine (ΔbasG), we sought to assess the contribution of endogenous and exogenous histamine to iron acquisition and siderophore production by *A. baumannii*. We find that disruption to histamine biosynthesis inhibits bacterial growth under iron restriction and on human serum, transferrin, and lactoferrin as sole sources of the essential metal. In the absence of endogenously synthesized histamine, exogenous addition of the amine restores the iron-dependent growth of and siderophore production by *A. baumannii*. These results suggest that the bacterium may be able to sense and utilize host-derived histamine to facilitate its iron-dependent growth *in vivo*. Together, it appears that histamine plays a complex and multifactorial role in the survival and pathogenesis of *A. baumannii*.

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Abstract 2717

Establishing the role for a putative dioxygenase in the stress response mechanism of *Leishmania donovani*

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Leishmania are parasitic protozoa that infect humans and their domestic animals and afflict millions of people worldwide. Currently, treatment approaches for *Leishmania* rely on just a few drugs that have poor safety profiles and for which their efficacy has been undermined by the development of drug resistance. Hence, there is a pressing need for new and better therapeutics for resolving *Leishmania* infections. Stress response pathways offer an attractive therapeutic paradigm for exploration, since they protect parasites from fluctuations in the host environment and promote parasite adaptation and survival. Previous studies from our laboratory used purine starvation (a type of nutrient stress encountered within the insect host) paired with quantitative proteomics to identify putative stress response candidates. Amongst the upregulated candidates, a putative dioxygenase has subsequently been identified, whose upregulation at both the mRNA and protein level appears to align with parasite adaptation and survival from purine starvation. The objective of these studies was to establish whether the putative dioxygenase is an integral part of the stress response mechanism in *Leishmania*. qRT-PCR analyses demonstrated that mRNA corresponding to the putative dioxygenase is upregulated in response to both nutrient and oxidative stress suggesting a broad role in the parasite response to stress. Likewise, overexpression of the putative dioxygenase protein conferred an increased resistance to the antileishmanial drug miltefosine. However, deletion of both copies of the putative dioxygenase gene from the *Leishmania donovani* genome by a CRISPR-Cas9 mediated strategy did not impact the ability of the resulting null mutant to survive nutrient stress, as well as other types of stress, suggesting that there is likely functional redundancy in the parasite response to stress. Furthermore, deletion of the putative dioxygenase gene did not adversely affect the growth of the resulting null mutants in culture, and, in contrast, led to significantly enhanced growth kinetics and metabolic activity in both the promastigote (insect) and amastigote (mammalian) life stage. Thus, we conclude that the upregulation of the putative dioxygenase while a hallmark of the response to various stressors encountered during the parasite lifecycle, is not essential for the survival of stress in *Leishmania*.

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Abstract 2718

Lactobacillus plantarum shows Probiotic Potential and Antioxidant properties for the Prevention of Colorectal Cancer

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Anasuya Roychowdhury

Colorectal cancer (CRC) emerges as a global threat. The commensal intestinal microflora maintains an equilibrium with the opportunistic pathogens in the gut to enrich the host immunity and metabolism. However, environmental factors and lifestyle behaviors often damage the protective epithelial mucosa. Imbalance in gut microbiota (dysbiosis) promotes chronic inflammation and triggers CRC. Probiotics are live microorganisms with health benefits on the host when consumed in adequate amount. Therefore, modulation of gut microbiome by consumption of probiotics could be a promising strategy for CRC prevention. Our published meta-analysis indeed rationalizes the probiotic implementation in the prevention of CRC and improvement of therapeutic benefit for CRC patients. However, characterization of novel probiotic strains with their potential anti-CRC mechanisms is the prime step for implementation of the strategy. In this study, we have performed bioinformatics analysis to explore the systematic network and identify the promising probiotic strains followed by elucidation of their anti-CRC effects with experimental (both bacterial as well as cell culture based) studies. Our data-mining approach identifies 27 anti-CRC probiotic bacteria and 135 genes from the analysis of 352 clinical, pre-clinical, *in-vitro*, and *in-vivo* data associated with CRC patients exposed to probiotic treatment. The potential probiotic strains selected by the systematic network analysis are then characterized for probiotic properties using *in-vitro* assays. We find, *Lactobacillus plantarum* ARC1 shows significant antimicrobial activity against enteric pathogens (including the multi-drug resistant strains). Characterization of other probiotic attributes shows that *L. plantarum* ARC1 can survive in acidic pH and high bile concentration, indicating its sustainability in the gut. Auto-aggregation assay signifies high potential of the strain for the colonization in the gut. Our adhesion assay with CRC cell line further endorses adherence property of *L. plantarum* ARC1 in the gut. Finally, the susceptibility of *L. plantarum* ARC1 to all conventional antibiotics confirms the absence of antibiotic-resistant genes. Prebiotic oligosaccharides function as food products of beneficial microbiome in the gut which remain non-digestible through the host digestive system. The prebiotic score was considerably high for fructooligosaccharides (FOS) and maltodextrin with *L. plantarum* ARC1, signifying a promising effect of the prebiotics. Moreover, the acidification profile (pH) was also significantly low, indicating the generation of short-chain fatty acids (SCFAs) by *L. plantarum* ARC1 in presence of prebiotics. The prebiotic index of *L. plantarum* ARC1 in presence of different doses of the prebiotics shows its dose-dependent growth pattern. Thus, the synergistic effects of

probiotics and prebiotics could drive them as an essential strategy to maintain healthy gut and prevent gut-inflammations. Interestingly, *L. plantarum* ARC1 shows remarkable free radical scavenging capacity indicating high antioxidant activity of the strain. Our network-analysis further identifies autophagy, intrinsic apoptotic pathway, stress-induced metabolic processes (JNK, MAPK), and immunomodulatory pathways as the major contributors. In conclusion, *L. plantarum* ARC1 is a promising probiotic with high anti-oxidant potential and could be further recognised as biotherapeutics for CRC. However, *in vivo* studies will reaffirm the application merit of *L. plantarum* ARC1 in CRC patients.

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Abstract 2720

Mechanistic Role of Intestinal specific Jak3 in Obesity-Associated Cognitive Impairments

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Background and aims: A compromise in intestinal mucosal functions is associated with several chronic inflammatory diseases. Previously, we reported that obese humans have a reduced expression of intestinal Janus kinase-3 (Jak3), a non-receptor tyrosine kinase, and a deficiency of Jak3 in mice led to predisposition to obesity-associated metabolic syndrome. Since meta-analyses show cognitive impairment as co-morbidity of obesity, the present study demonstrates the mechanistic role of Jak3 in obesity associated cognitive impairment. Our data show that high-fat diet (HFD) suppresses Jak3 expression both in intestinal mucosa and in the brain of wild-type mice. **Methodology:** Recapitulating these conditions using global (Jak3-KO) and intestinal epithelial cell-specific conditional (IEC-Jak3-KO) mice and using cognitive testing, western analysis, flow cytometry, immunofluorescence microscopy and 16s rRNA sequencing, we demonstrate that HFD-induced Jak3 deficiency is responsible for cognitive impairments in mice, and these are, in part, specifically due to intestinal epithelial deficiency of Jak3. **Results:** We reveal that Jak3 deficiency leads to gut dysbiosis, compromised TREM-2-functions-mediated activation of microglial cells, increased TLR-4 expression and HIF1-α-mediated inflammation in the brain. Together, these lead to compromised microglial-functions-mediated increased deposition of β-amyloid (Aβ) and hyper-phosphorylated Tau (pTau), which are responsible for cognitive impairments. Collectively, these data illustrate how the drivers of obesity promote cognitive impairment and demonstrate the underlying mechanism where HFD-mediated impact on IEC-Jak3 deficiency is responsible for Jak3 deficiency in the brain, reduced microglial TREM2 expression, microglial activation and compromised clearance of Aβ and pTau as the mechanism during obesity-associated cognitive impairments.

Conclusion: Thus, we not only demonstrate the mechanism of obesity-associated cognitive impairments but also characterize the tissue-specific role of Jak3 in such conditions through mucosal tolerance, gut-brain axis and regulation of microglial functions.

Discovery Foundation.

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Abstract 2722

Mechanisms of host adhesion and biofilm formation mediated by the staphylococcal surface protein Aap

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Staphylococcus epidermidis is a gram-positive commensal bacterium that routinely colonizes the skin, where it generally plays a beneficial role by helping to control invasion by pathogens. However, *S. epidermidis* is also the leading cause of device-related infections after surgery, due to its strong propensity to form biofilms on implanted devices. Biofilms are surface-adherent clusters of bacteria that not only show strong adhesion and cohesion, but also render the bacteria within resistant to antibiotic action or host immune responses. Remarkably, both host adhesion and biofilm formation by *S. epidermidis* are mediated by the same staphylococcal surface protein called Aap. The objective of our work is to use a combination of structural, biophysical, and microbiological assays to reveal the mechanisms of these disparate functions mediated by Aap. A lectin domain in the N-terminal portion is responsible for adhesion to healthy human corneocytes. A high-resolution crystal structure revealed a legume lectin-like fold with atypical binding site architecture. Glycan array data combined by isothermal titration calorimetry identified glycan ligands for Aap and structure-guided mutagenesis confirmed the glycan binding site in the lectin domain, providing insights into ligand specificity and interspecies competition for colonization of the skin niche. Alternatively, the lectin domain of Aap can be removed by the protease SepA, unmasking the B-repeat superdomain and allowing intercellular adhesion and biofilm formation. The B-repeat region self-assembles in the presence of Zn2+, forming first dimers and then tetramers or higher-order oligomers, depending on strain-dependent variations in the sequence of the B-repeat superdomain. Both tetramers and higher-order oligomers undergo a conformational change at physiological temperature to nucleate functional amyloid fibers within the nascent biofilm. Understanding the nature of the B-repeat assemblies will help to provide insights into amyloid nucleation and lay the groundwork for potential therapeutic interventions.

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Abstract 2728

Inducible Cas9 and Overexpression Systems for Study of Gene Function and Regulation Across Malaria Lifecycle

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Plasmodium falciparum, the parasite responsible for the deadliest form of malaria, has a complex lifecycle involving various host cell environments in both human and mosquito hosts. With only ~5500 genes, the parasite must tightly regulate gene expression at each stage in order to adapt to its current environment while continuing development. Without continuous culture outside of the asexual blood stage, it is challenging to study gene function and regulation in subsequent stages *in vitro*. Knocking out genes essential to the asexual blood stage prohibits further study in other stages. Thus, we adapted a recently developed dimerizable Cre recombinase system for leak-free controllable over-expression and Cas9-directed knockouts. Before rapamycin is added, GFP is expressed and the gene of interest is not expressed. After rapamycin, the GFP is floxed out, and the promoter drives expression of the gene. Using Cas9 under this inducible system, we can perform HDR editing conditionally, turning it on at specific timepoints or lifecycle stages. These tools, combined with the previously developed DiCre-CRISPRa and CRISPRi systems, allow for temporal over-expression, under-expression, and knockout for gene characterization across multiple timepoints and developmental stages. We recently developed a genome-wide gene regulatory network model of *P. falciparum* and discovered that parasite populations that are transcriptionally heterogenous showed increased resistance to artemisinin sensitivity, likely a result of a diversified population. To better understand and characterize this network and transcriptionally induced diversity, we sought to use our inducible systems to over-express and conditionally knock out transcriptional regulators in both asexual and gametocyte stages. Future experiments involve transcriptomic characterization following knock-out and over-expression, as well as expanding these systems to study multiple genes and transcriptional regulators simultaneously. In conclusion, we have developed systems for conditional over-expression and Cas9 knockout that allow for accessible characterization of gene function across the lifecycle.

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Topic Category Chemical Biology, Drug Discovery and Bioanalytical Methods

Abstract 138

Acylspermidines are conserved mitochondrial sirtuin-dependent metabolites

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Sirtuins are NAD+-dependent protein lysine deacylases that play a central role in the regulation of metabolism and stress responses. However, identification of acyl groups removed by different sirtuins *in-vivo* has remained incomplete, and the downstream metabolic fate of removed acyl moieties is unclear. Using untargeted comparative metabolomics, chemical synthesis, and multiple HPLC chromatographic techniques, we identified N-glutarylspermidines (previously uncharacterized small molecules) as metabolites downstream of the mitochondrial sirtuin sir-2.3 in *C. elegans*. Parallel comparison of mitochondrial protein acylation levels revealed increased lysine glutarylation in sir-2.3 knockout mutants by a tandem mass-spectrometry based method and western blot, indicating that SIR-2.3 functions to deglutarylate lysines, which was further supported by molecular modeling. We further show that N-glutarylspermidines can be derived from non-enzymatic acyl transfer from O-glutaryl-ADP-ribose, the direct product of sirtuin-mediated deglutarylation, onto spermidine. Identification of N-glutarylspermidines as deacylation-derived metabolites enabled tracing the origin of the sirtuin-derived glutaryl moieties to catabolism of free lysine, and led to the finding that in sir-2.3 mutants lysine supplementation results in developmental delay, likely as result of an increased mitochondrial protein glutarylation. Targeted analysis of *C. elegans*, mouse, and human metabolomes revealed a range of N-acylspermidines corresponding to most known N-acyl substrates of sirtuins. We also observed formation of N-succinyl- and/or N-glutarylspermidines downstream of mammalian mitochondrial SIRT5/Sirt5 in two cell lines, consistent with increased lysine succinylation and glutarylation levels we detected in SIRT5/Sirt5 knockout cells. Our results indicate that N-acylspermidines represent a conserved metabolite family downstream of mitochondrial sirtuins that facilitate annotation of sirtuin enzymatic activities *in-vivo*. This work demonstrates that untargeted comparative metabolomics provides the opportunity to associate new or unexpected metabolites with specific pathways, therefore having the potential to reveal "missing links" in important pathways.

This work was partly supported by the NIH (R35 GM131877 to F.C.S; R01 CA223534 to R.S.W; P40 OD010440 to Caenorhabditis Genetics Center; GM69702 to APBS-PDB2PQR software). I.R.F was supported by HHMI Gilliam Fellowship GT11525, and J.M was supported by NIH grant F30 CA250451.

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ASBMB

Abstract 144

Bioactivity of Atlantic Stingray, Hypanus sabinus, venom on murine cell line

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Cathy Walsh, Carl Luer

Venoms are a complex concoction of compounds that have proven to be useful as molecular tools and therapeutic agents. Stingray venom presents this same potential, but has not been well studied since venom glands on stingray spines exist as diffuse tissue rather than as distinct organs. The objective of this preliminary study was to characterize a variety of biological activities of venom compounds from the Atlantic stingray, Hypanus sabinus, on a target cell line (murine fibrosarcoma, WEHI 164). Biochemical assays included an MTT assay to determine effects on cell growth, a cell-based assay to determine production of reactive oxygen species, and a cell flow cytometry-based assay to determine apoptosis/necrosis in treated cells. Protein profiles of venom gland tissue collected from both ventral and dorsal surfaces of spines were assessed with SDS-PAGE gels stained with Coomassie Blue or silver stain. Gels stained in Coomassie Blue showed no difference between ventral and dorsal surface proteins. Two additional bands visible with silver stain will require further characterization to determine their significance. Results from the flow cytometry assay using annexin V to determine apoptosis were preliminary and inconsistent. One trial showed an increase in necrosis while others showed no difference from untreated samples. There was also no difference in apoptotic cytotoxicity between ventral and dorsal samples with increasing protein concentrations (0–20 ng/mL). Parameters for venom extraction need to be better defined to ensure reproducibility of protein preparation for assays before characterization of venom bioactivity can be confirmed.

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Abstract 154

Inhibition of *Staphylococcus aureus* SsbA by the stem extract of the pitcher plant *Sarracenia purpurea*

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Cheng-Yang Huang

Staphylococcus aureus, a major threat to public health, exhibits a remarkable ability to develop antibiotic resistance and causes more than 20 000 deaths per year in the United States. The *S. aureus* SsbA protein (SaSsbA) is a single-stranded DNA-binding protein that is categorically required for DNA replication and cell survival, and it is thus an attractive target for potential antipathogen chemotherapy. In this study, we prepared the stem extract of *Sarracenia purpurea* obtained from 100% acetone to investigate its inhibitory effect against SaSsbA. *S. purpurea* is a carnivorous pitcher plant and exhibits many ethnobotanical uses. Initially, myricetin, quercetin, kaempferol, dihydroquercetin, dihydrokaempferol, rutin, catechin, β-amyrin, oridonin, thioflavin T, primuline, and thioflavin S were used as possible inhibitors against SaSsbA. Of these compounds, dihydrokaempferol and oridonin were capable of inhibiting the ssDNA-binding activity of SaSsbA with respective IC50 values of 750 ± 62 and 2607 ± 242 μM. Given the poor inhibition abilities of dihydrokaempferol and oridonin, we screened the extracts of *S. purpurea, Nepenthes miranda*, and *Plinia cauliflora* for SaSsbA inhibitors. The stem extract of *S. purpurea* exhibited high anti-SaSsbA activity, with an IC50 value of 4.0 ± 0.3 μg/mL. The most abundant compounds in the stem extract of *S. purpurea* (>4.7%) identified using gas chromatography–mass spectrometry were driman-8,11-diol, deoxysericealactone, stigmast-5-en-3-ol, apocynin, and α-amyrin. Using the MOE-Dock tool, the binding modes of these compounds, as well as dihydrokaempferol and oridonin, to SaSsbA were elucidated, and their binding energies were also calculated. The binding capacity of these compounds was in the following order: deoxysericealactone > dihydrokaempferol > apocynin > driman-8,11-diol > stigmast-5-en-3-ol > oridonin > α-amyrin. In addition, we also found that the clinical anticancer drug 5-fluorouracil (5-FU) was capable of binding to SaSsbA. We further cocrystallized 5-FU and SaSsbA and solved the complexed crystal structure (PDB ID 7YM1) to determine where binding occurred and investigated the binding mode. Overall, we hope that these preliminary findings may facilitate the development of new inhibitors to target SaSsbA for further clinical antipathogen chemotherapy.

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Abstract 161

Spatiotemporally heterogeneous nature of γ-secretase in live/intact neurons

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γ-Secretase is a membrane-embedded aspartyl protease complex responsible for the proteolytic processing of a wide variety of transmembrane proteins such as APP and Notch receptors. While some γ-secretase cleavage products function as signaling (e.g., Notch ICD) or pathogenic molecules (e.g., β-amyloid), others may be intermediates that are destined for degradation (i.e., γ-secretase may act as "the proteasome of the membrane"). γ-Secretase is widely expressed throughout the brain and plays a pivotal role in neurogenesis during development. Furthermore, dysfunctional γ-secretase is tightly associated with the progression of Alzheimer's disease (AD). Yet, little is known about how γ-secretase activity is spatiotemporally regulated, as there are no tools currently available to examine the dynamic nature of γ-secretase activity in living cells. To overcome this drawback, we have recently developed novel genetically encoded Förster resonance energy transfer (FRET)-based biosensors, which enable for the first time quantitative recording of γ-secretase activity, over time, on a cell-by-cell basis, in live neurons *in vitro* and mouse brains *in vivo*. Using the new biosensors, we uncovered that γ-secretase activity is heterogeneously regulated in individual neurons. Furthermore, in line with previous studies employing proteoliposomes *in vitro*, the cell-to-cell heterogeneity in γ-secretase activity is tightly associated with altered lipid membrane properties in live/intact neurons. Our biosensors would not only be valuable tools to identify the molecular regulators of γ-secretase activity but also provide previously missing capability to distinguish cell populations with different endogenous γ-secretase activity in live cells. Such ability would shed light on the mechanistic link between a change in γ-secretase activity and its biological consequences in normal physiology and diseases.

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Abstract 165

A bioinformatics approach to establish principles for designing modular transcriptional regulators

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Vincenzo Kennedy, Ala Hessami

Allosterically regulated transcriptional regulators are commonly used as genetic sensors for controlling cellular activities. However, each native regulator only responds to a unique molecular signal to control a specific promoter for gene expression; this lack of flexibility limits the development of genetic response to complex environmental changes. The objective of our work is to develop an efficient and robust method for designing modular regulators with programmable combinations of DNA recognition and allosteric response. Among LacI family regulators, we identified a DNA-binding module (DBM) and a ligand-binding module (LBM), which are responsible for interacting with DNA-based promoters and detecting signaling molecules, respectively. These modules are highly conserved and fusing different DBMs and LBMs can construct hybrid regulators that support new signal-genetic control connections. Furthermore, we have developed a computational predictive model based on coevolutionary cues from over 70 000 family members, which facilitated us to identify key interactions between the DBM and LBM. We also used this predictive model to design mutants to improve regulator performance. To demonstrate the potential of these hybrid regulators, they were used to implement several new genetic circuit designs that can lead to biotechnological applications. Overall, this work represents our approach for creating novel genetic tools and the use of these tools for developing unique decision-making behaviors in cellular systems.

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Abstract 1165

Potential "Release-on-Demand" Metallopolymer Coatings For Drug-Release, Biofabrication, and *In Vitro* Experimental Control

Jessica Schneider, *University of Dayton*

Justin Biffinger, Russell Pirlo

Dynamic or Smart biomaterials have proven effective in multiple biomedical applications including the controlled release of drugs, biofabrication of engineered tissue constructs like cell-sheets, and in the spatial and temporal release of biofactors within *in vitro* tissue models. The current and potential applications present an unmatched need for more 'release-on-demand' approaches and materials. We will present the synthesis and characterization of metal containing polyester coatings using piperazine in the backbone used to coordinate either Fe(III) or Ru(III) metal centers. The stability of these coatings were high when the metals were not present over 15 days but self-catalytically degraded when either iron or Ru was present. We then 3D printed polylactic acid condensed with the bis diol piperazine linkages to create polymer coatings that could potentially release artificial or mammalian-based tissues upon either an electrochemical or light stimulus. These coatings are applicable to a wide range of tissue engineering platforms for drug discovery.

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Abstract 1168

Purification of Recombinant Histidine-Tagged Lanmodulin without Size Exclusion Chromatography and Metal Binding Affinities for Eu(III) or Fe(III)

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Emily Koerner, Martha Carter, JD Gillum, Nancy Kelley-Loughnane, Chia-Suei Hung, Justin Biffinger

Selective precious metal sequestration and purification from mixed metal waste streams will be a sustainability challenge for the foreseeable future. Several fundamental and applied research studies have shown that the intrinsically disordered protein Lanmodulin (LanM), which is analogous to Calmodulin, can selectively bind rare earth metals at pM efficiency. Our research efforts to scale up the production of recombinant LanM from *E. coli* BL21(DE3) have experienced significant issues with the purification of LanM using Hist-tags on Ni-NTA and Co-NTA beads. Thus, an additional size exclusion chromatography (SEC) purification step needs to be included which is a time and resource intensive step when attempting to scale production of a protein. Our systematic approach to isolate high purity Hist-tag LanM directly from Ni-NTA column fractions without a size exclusion chromatography step was successful. We confirmed that LanM purified using this scalable SEC-free method had a purity between >97% (by densitometry gel imaging). We will also present complementary selective metal binding results for Eu (III) and Fe(III) with LanM using data from a colorimetric xylenol orange back-titration approach and calculation of metal binding efficiencies directly with ion chromatography.

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Abstract 1183

Saliva effect on RT-PCR performance

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The use of saliva directly in RT-PCR reactions may be interesting, particularly in screenings for the detection of DNA/RNA of pathogens, due to the advantages of time and costs. However, its use faces some difficulties that essentially result from the saliva being a matrix with a variable composition and which contains inhibitors of the polymerization reaction. Saliva samples obtained from healthy donors were spiked with SARS-CoV-2 RNA and 3 viral targets were detected by RT-PCR. This work was considered exempt from review by an institutional ethical review board, because it comprises use of completely anonymized specimens obtained voluntarily and informed. The efficiency of RT-PCR reactions was calculated in the presence of variable amounts of saliva and the effect of saliva pre-treatment on the performance of each reaction was also analysed. It was found that saliva has no significant influence on the mean efficiency of each individual reaction, affecting mainly the beginning of the exponential phase of amplification, resulting in a significant increase in Ct values. This effect which is target dependent, presents a highly between-person variability (ΔCt = 0.93 to 11.36) and affects RNA/DNA detection and the limit-of-detection, but can be partly reversed by a treatment of saliva prior to the reaction. The work presented contributes to a better understanding of the advantages and limitations of using saliva directly in RT-PCR.

This work was supported by ProjectNORTE-01-0145-FEDER-072545- SAICTCOVID/72545/2020 and was the result of the projects: UIDB/05937/2020 and UIDP/05937/2020—Centre for Research and Development in Agrifood Systems and Sustainability—funded by national funds, through FCT-Fundação para a Ciência e a Tecnologia.

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Abstract 1187

A Quantitative Comparison of the Knockdown Efficiencies of CRISPR/Cas9 and CRISPR-Cas12 in *Caenorhabditis elegans*

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CRISPR is an invaluable genetic engineering tool for understanding the mechanisms of diseases, drug resistance, and single-cell biology (Yan & Wang, 2019). CRISPR contains a crRNA, or CRISPR targeting RNA, a 20 nucleotide sequence from the spacer region that forms an RNA duplex with a trRNA that directs the Cas9 endonuclease to the target site (Kim & Colaiácovo, 2019). While the functionality of CRISPR/Cas9 has been further elucidated in the past several years, there are numerous limitations, one being the low gene knockdown efficiency (Bandyopadhyay et al, 2020). CRISPR-Cas12, on the other hand, is hypothesized to have a higher editing efficiency. It also creates staggered ends rather than blunt ends in the DNA, leading to fewer mistakes during re-ligation (Yan & Wang, 2019). Despite these differences in the mechanisms of the two systems, no studies have directly compared CRISPR/Cas9 and CRISPR-Cas 12 in organisms other than plants. Through bacterial transformation, we expressed our modified plasmid (containing the gRNA sequence, Cas endonuclease sequence, and *C. elegans*-specific promoters) in *Escherichia coli*. The *C. elegans* subsequently ingested the transformed bacteria. Fluorescence and protein expression were used as assays to determine the efficacy of CRISPR-mediated GFP deletion. We intend to quantitatively assess the difference in loss of function efficiencies between CRISPR-Cas9 and CRISPR-Cas12 when used to delete GFP in *C. elegans*. Our goal is for these findings to be valuable for researchers seeking to use the most efficient Cas endonuclease for gene knockdown experiments.

This research was funded by The Nueva School. The Shen Lab at Stanford University provided access to equipment, including a fluorescence microscope. LP162 *C. elegans* were provided by the Caenorhabditis Genetics Center at the University of Minnesota. Lastly, thanks to Luke De and Paul Hauser, the primary mentors for this project, for general project advice.

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ASBMB

Abstract 1188

Codon Optimization of saCas9 Gene Enhances Protein Expression in Human Kidney Cells to Compensate for Difficulties in Delivery Vectors to Boost CRISPR-Cas9 *in vivo* Therapeutic Application

Anoushka Krishnan, *The Nueva School*

Zander Chierici, Dhruv Chinmay, Hannah Drew, Eli Cooper, Benjamin Cheng, Luke De, Paul Hauser

The CRISPR-Cas9 system is a novel gene editing technology with the ability to alter target sequences in the genome dictated by a guide sgRNA. This technology has huge potential in treating genetic diseases, but *in vivo* CRISPR-Cas9 editing is currently mostly infeasible. One factor why *in vivo* CRISPR-Cas9 editing is infeasible is low Cas9 expression efficiency. The large size of the Cas9 gene (~4500 bp) inhibits delivery by the small viral vectors and lipid nanoparticles used. This study aims to observe the impact of codon optimization on the protein expression of the Cas9 plasmid in HEK293t cells. We hypothesize that a codon-optimized Cas9 gene that supports significantly higher protein expression than the presently used genes can compensate for the low delivery efficiency of either viral vectors or LNP-mRNA. In addition, these results could significantly improve the *in vivo* efficiency of the CRISPR-Cas9 system. We selected a commonly used parent saCas9 sequence from Addgene's public plasmid database, and used it to develop a novel codon-optimized gene sequence using Genscript's OptimumGene™ algorithm. To test our hypothesis, we will compare the protein expression of our optimized saCas9 plasmid with 3 commercially available plasmids via Western Blot analysis. Previous Western Blot and ELISA data suggests that the optimized sequence exhibits on average a 3.5 fold increase in Cas9 protein expression compared to the commercially available plasmids.

This research was funded by The Nueva School. The Shen Lab at Stanford University also provided access to HEK293t cells, reagents and necessary equipment. Plasmids were supplied by Addgene, the Feng Zhang laboratory at MIT, the Keith Joung laboratory at Harvard, and the Kiran Musunuru lab at the University of Pennsylvania.

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Abstract 1190

Adding DNA Binding Domains: A New Strategy for Improving XNA Polymerases

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The utility of unnatural nucleic acids, Xeno Nucleic Acids (XNA), enhances DNA's biotechnological and biomedical applications due to improved properties such as nuclease resistance. However, natural DNA polymerases cannot synthesize XNA; thus, XNA polymerases have been identified through directed evolution that allow for the enzymatic synthesis of XNA. However, the synthesis of XNA by XNA polymerases remains far less efficient and accurate than natural DNA synthesis, limiting XNA applications. To overcome this challenge, we hypothesize that fusing DNA Binding Domains to engineered XNA polymerases may further improve the processivity and affinity of XNA polymerases. Here, we describe the successful fusions of leading XNA polymerases with DNA binding domains. We developed a modular cloning strategy that allowed us to generate 11 combinations of XNA polymerases and DNA binding domains. Expression and purification of these engineered proteins required substantial optimization as the enzymes are likely toxic to commonly used protein-producing *E. coli* cell lines. We also present preliminary data showing changes in XNA binding as well as potential use in PCR. The application of XNA Binding Domain fusions extends the possibility of a rapid and modular strategy for improving XNA polymerases to enable new applications in XNA that require more efficient synthesis.

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Abstract 1192

Mapping small molecule binding pockets on lipid kinases using chemoproteomics and AlphaFold

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Diacylglycerol kinases (DGKs) are metabolic kinases involved in regulating cellular levels of diacylglycerol and phosphatidic lipid messengers. The development of selective inhibitors for individual DGKs has been hindered partially by a lack of known protein pockets available for inhibitor binding in cellular environments. This study utilized a DGK-directed sulfonyl-triazole probe to modify tyrosine and lysine sites on DGKs in cells in order to map predicted small molecule binding pockets in AlphaFold structures. We apply this integrated chemoproteomics and AlphaFold approach to evaluate probe binding of DGK chimera proteins engineered to exchange regulatory C1 domains between DGK subtypes (DGKA and DGKZ). Specifically, we discovered alterations in TH211 binding in the catalytic domain when C1 domains on DGKa were exchanged that correlated with impaired biochemical activity as measured by a DAG phosphorylation assay. In summary, we provide a family-wide ligand binding map of reactive sites that combined with AlphaFold revealed predicted small molecule binding pockets for guiding future inhibitor development of the DGK superfamily.

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Abstract 1198

Machine-Learning Assisted Residue Optimization of Laccases for Dye Decolorization

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Peter Choi, Owen Hsu, Paul Hauser, Luke De

Modern industrialization has led to the development of many practices that disperse environmentally novel substances, such as crude oil, pesticide, herbicide, insecticide, plastic, and dyes into vital ecosystems which, as a result of their environmental novelty, accumulate, often creating negative externalities. The bacterium *Bacillus safensis* contains a cell free laccase capable of decolorizing dye which has applications as a bioremediation enzyme; however, it denatures in pHs typical working environments such as bodys of water with a pH greater than 6.5. To combat this inability to function in preferred environmental conditions, screening procedures are commonly used. These methods are unfavorable because they are costly and rarely yield promising results. In this study, we explore a more efficient method for the advent of robust bioremediation enzymes: the leveraging of the unprecedented ability to predict protein structure from language data and Mutcompute, a structure driven machine learning approach to enzyme residue optimizations. These tools can be used to aid the engineering of more robust enzyme variants capable of better function in their working environments. This research aims to mutate the cell free laccase of the *Bacillus safensis* according to the MutCompute predicted mutations and compare the mutant enzyme activity with the wild type enzyme activity. Furthermore, we present a pipeline for protein engineering research in the highschool setting using relatively inexpensive equipment along with laccase mutant sequences for pH optimization as well as verification that pHs greater than 6 causes the denaturation of the wild type cell free laccase of the *Bacillus safensis*.

Our project is part of the eXpirement Research Teams department of The Nueva School.

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Abstract 1199

Discovery of red-shifting mutations in firefly luciferase using high-throughput biochemistry

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Nicole Oberlag, Anjali Mawidmar, Edison Reid-McLaughlin, Lyndsey Flanagan, Synaida Maiche, Aaron Leconte

Firefly luciferase is a bioluminescent enzyme which produces light via an oxidative reaction with its substrate, luciferin. The light produced by luciferase can be used in bioluminescent imaging (BLI) to visualize the movement and growth of cells over time in live tissue. While this is a valuable tool with vast biomedical implications, the natural wavelength of light emitted by *Photinus pyralis* luciferase is largely absorbed by endogenous biomolecules in tissue, limiting the brightness and accuracy of signal emitted by the enzyme in BLI. Mutations to *P. pyralis* luciferase can shift the emission spectra from yellow-green to red, a more favorable color for BLI as its spectrum is more easily visualized in deep tissue. There are several theories which could explain this phenomenon, however, the exact mechanism contributing to the observed color changes in mutated luciferase enzymes is largely disputed. Here, we have used deep mutational scanning at 20 amino acid positions identified by statistical coupling analysis. Each has been assessed for activity and the emission colors of all mutants. This approach allowed us to identify several positions which contribute to red shifting when mutated, as well as distinguish highly mutable positions from more strongly conserved residues. Though some of the positions identified by statistical coupling analysis have been previously shown to cause red-shifting, the majority have been largely unexamined in this context, yielding promising new results and novel color shifting candidates. By collecting pools of red-enriched mutants and analyzing them via high throughput sequencing, the specific identities of red-shifting mutants will be identified and can be used for more effective bioluminescent imaging. This high throughput approach contributes to the development of a comprehensive sequence-function map of the luciferase protein and broadens our understanding of how the structure of luciferase dictates its function.

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Abstract 1204

Assessing the Scope and Accuracy of 2' Az modified XNA Synthesis

Hailey Kang, *Claremont McKenna College*

Ananya Venkatesh, Aaron Leconte

Nonnatural DNA (xeno nucleic acids, XNA) can have valuable biotechnological applications due to its increased nuclease stability and ability to incorporate new nonnatural functional groups. As natural DNA polymerases are not able to synthesize XNA, mutant XNA polymerases have been created that can synthesize some forms of XNA; however, the full extent of the types of XNA that can be synthesized is still being discovered. Here, we assess both the extent and accuracy that 2' Az XNA can be incorporated in mixed polymers with other XNAs. We chose to focus on azides as they introduce new functionality via facile chemical labeling using click chemistry. To investigate, leading Taq DNA polymerase mutants (SFM4-3, SFM4-6, SFP1) were utilized to determine the scope of XNA synthesis of mixed polymers containing azides and other 2' modifications; we found that SFM4-3, SFM4-6, and SFP1 readily synthesize mixed polymers of 2' Az XNA with RNA and 2' F XNA, but cannot synthesize mixed polymers containing 2' FANA XNA. To further assess the synthesis of 2'Az/2'F mixed polymers, high-throughput sequencing was used to determine the synthesis accuracy. This study expands the types of XNA into which azides can be incorporated, allowing for further modification of XNAs using click chemistry.

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Abstract 1205

Using High Throughput Biochemistry to Identify Functionally Important C-terminal Mutants in Firefly Luciferase

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The mutagenesis of firefly luciferase has increased the capabilities of bioluminescent imaging and led to the creation of sets of luciferase pairs capable of multicomponent imaging. Bioluminescent imaging *in vivo* requires red-shifted luminescence in order to achieve better resolution through layers of tissue. The development of improved and red-shifted luciferases relies on a deeper understanding of understudied domains of the enzyme. To this end, we utilized alanine scanning, a high throughput biochemical approach, to characterize the C-terminal domain of firefly luciferase. Libraries derived from the mutation of each individual residue in the C-terminus to alanine were screened for changes in enzyme activity and luminescence emission color. A multiple stage screening process determined eight residues that showed red-shifted emissions multiple times. Libraries of all twenty amino acids at each of the eight locations were created to further optimize luminescence color. Preliminary data showing novel red-shifting amino acid changes is presented. This work expands our understanding of how emission color is determined and highlights the importance of using high throughput techniques for characterizing large swaths of understudied regions of the luciferase protein.

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Abstract 1216

Thermal Stability of Animal Orthologs of the Lysosomal Protease Tripeptidyl-peptidase 1 Does Not Correlate with Cellular Half-life: Implications for Evolutionary Redesign of the Human Enzyme

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Whitney Banach-Petrosky, David Sleat, Peter Lobel

Introduction: We previously reported that the thermal stability of human lysosomal proteins does not correlate with cellular half-life. However, similar studies for a variety of other enzymes concluded that increased thermal stability is associated with increased resistance to protease digestion, which yield increased cellular half-life. Improving the *in vivo* stability of tripeptidyl-peptidase 1 (TPP1) would be advantageous in treating late-infantile neuronal ceroid lipofuscinosis (LINCL), a neurodegenerative lysosomal storage disease (LSD) caused by a deficiency of TPP1. Given recent efforts at evolutionary redesign of lysosomal enzymes to treat LSDs such as metachromatic leukodystrophy, we sought to determine if the trends observed for human lysosomal proteins existed across orthologs in the animal kingdom and to assess the potential application of evolutionary redesign for improving the existing enzyme replacement therapy (ERT) for LINCL.

Methods: The human TPP1 protein sequence was searched against all known animal (TAXID: 33208) orthologs in the NCBI non-redundant protein sequences (nr) database using the BLASTP algorithm. "Predicted" and "low quality" protein sequences were excluded. Orthologs from each class of phylum Chordata were selected. The protein sequences were converted to codon-optimized gene fragments with C-terminal hexa-histidine (C-His) tags and cloned onto an N1 plasmid vector for expression in Chinese hamster ovary (CHO) cells. Proteins were purified from media and assayed for enzymatic activity, *in vitro* thermal stability at 60°C, and *in vivo* cellular half-life. Enzymatic activity was normalized against wild-type human TPP1C-His. Thermal and cellular half-lives were compared to that of wild-type human TPP1C-His using one-way ANOVA and the resulting p-values were adjusted for multiple comparisons using Dunnett's T3 multiple comparison test.

Results: Ten orthologs were synthesized, of which nine expressed at a level sufficient for purification. Wild-type human TPP1C-His was determined to have a mean thermal stability of 4.8 minutes (95% CI: [3.1, 6.6]) at 60°C and a mean cellular half-life of 52.9 (46.1, 59.8) hours. TPP1 orthologs from *Rattus norvegicus* (13.8 min [13.3, 14.3], $p < 0.0001$), *Heterocephalus glaber* (10.3 min [9.5, 11.0], $p = 0.0031$), *Danio rerio* (12.0 min [10.5, 13.4], $p = 0.0009$), and *Gallus gallus domesticus* (11.4 min [10.8, 12.0], $p = 0.0013$) exhibited increased thermal stability, while TPP1 orthologs from *Xenopus laevis* (1.2 min [0.8, 1.6], $p = 0.0227$) and *Bos taurus* (0.7 min [−1.8, 3.2], $p = 0.0083$) exhibited reduced thermal stability at the 0.05 significance level.

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There were no statistically-significant differences in cellular half-life compared to wild-type human TPP1C-His at the 0.05 level of significance.

Conclusion: Increases in *in vitro* thermal stability do not correlate with increases in *in vivo* cellular half-life among the nine tested TPP1 orthologs, which broadly represent kingdom Animalia. Our findings are consistent with previous findings on the correlation between thermal stability and cellular half-life of TPP1 in particular and lysosomal enzymes in general. These data suggest that TPP1 may not be suitable for evolutionary redesign and that there is limited scope for improving the current ERT for LINCL, although further testing of the kinetic properties of each ortholog is required.

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Abstract 1225

Wrangling the potential of lasso peptides as a molecular scaffold: comparisons with cyclic peptide inhibitors of thrombosis initiation

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Peptides are full of untapped potential as novel therapeutics. Those peptides with specific structural constraints often fare well in the cellular environment. With safer profiles, and stable structures linked to unique functions, constrained peptides can inhibit many protein-protein interactions involved in disease. Heart attacks and strokes from associated cardiovascular disease remain the top health concerns in America. In arterial thrombosis, the blood protein von Willebrand Factor (vWF) often initiates clots by binding to collagen, prior to the recruitment of platelets. By targeting this first step in thrombosis using peptide-based inhibitors, safe, stable and potent compounds are achieved. We have established methods for creating head-to-tail cyclic peptides with a precedent for inhibiting vWF binding to collagen with moderate activity and exceptional proteolytic stability. To improve on these results, we have continued work on examining the use of non-natural aromatic amino acid substitutions in key places along our cycles. In addition, efforts have been made to engineer lasso peptides through site-directed mutagenesis to display an important motif grafted from our cyclic peptides on their constrained loop. Lasso peptides are a class of antimicrobial natural products produced from bacteria by a conserved gene cluster that dictates the folding of their unusual topology, processing and export from the cell. Our lasso peptides have been altered to contain a sequence that competes with vWF for binding collagen as determined by our published fluorescence-linked immunosorbent assay ("FLISA"). Additionally, all of our peptides are rigorously tested by a panel of specific proteases over extended times, demonstrating immense stability when compared to linear counterparts. We found that a biologically engineered lasso peptide was a much stronger inhibitor of the vWF-collagen interaction when compared to our cyclic peptide counterparts. The lasso peptides also proved more soluble and had analogous stability to the cyclic peptides previously examined. Peptides have thus far proven their worth in inhibitor development targeting the protein-protein interaction that initiates thrombosis. This work informs future designs and further testing in the improvement of peptide-based anti-thrombosis drugs regarding molecular shape, peptide sequence, and the pathway to compound development.

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Abstract 1244

Optimization of PCR Using XNA Nucleotides

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Trevor Christensen, Aaron Leconte

DNA with modifications to either the base pairs or the sugar-phosphate backbone (xenonucleic acids, XNA) can improve nuclease stability, allow the molecule to engage in new chemical reactions, and more. Currently, XNA synthesis is a time-consuming process that can only produce shorter strands of XNA. A possible improvement to this issue is finding a method to PCR amplify XNA. Previous papers have shown that some mutant versions of DNA polymerase I from Thermus aquaticus (Taq) is able to PCR amplify molecules using a nucleotide mixture of one modified NTP (2'F) and three normal dNTPs. It is known that modified versions of Taq Polymerase are able to synthesize XNA from and XNA template; however, there is no evidence that current enzymes are able to synthesize XNA using an XNA template. Before we can progress in terms of developing new enzymes, as base line of what is currently possible needs to be established. I have since been able to show that serval of the known mutant Taq polymerases are PCR active and can perform PCR using the nucleotide mixes of one 2'F NTP and three dNTPs. Not only can these mutations perform PCR using one 2'F NTP, but many of these polymerases can also perform PCR using two modified nucleotides and two normal dNTPs. This means we may be closer to the full PCR amplification of XNA than previously believed.

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Abstract 1251

Progress Toward Directed Evolution of a Nanobody to Bind Nipah Virus G-Protein

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Rayna Edwards, Craig Streu

Nipah virus is a zoonotic virus that originates in fruit bats in Southeast Asia. It is highly contagious and easily transmissible, so much so that the World Health Organization has listed it as one of the top ten greatest risks of the next epidemic. There are currently no available vaccines or treatments, and the death rate is 40 to 100 percent. Structurally, Nipah virus's mechanism of infection is through its surface glycoprotein (G-protein), a transmembrane protein with a large extracellular domain that recognizes and binds to receptors on the surface of host cells. This is the first step in the virus's invasion pathway, followed by entry of the virus into the host cell by endocytosis, and then release and replication of the viral contents. Our project is focused on finding a nanobody to bind to Nipah virus's G-protein, inhibiting its ability to bind to host cells and therefore cutting the invasion pathway off at the first step, which in theory will completely prevent an infection from occurring. Herein, we outline progress toward the selection of nanobody binders of the extracellular fragment of Nipah G-protein. Since nanobodies lack the highly immunogenic conserved regions of full antibodies, they hold potential for the treatment of both humans and animals. As such, a successful nanobody treatment can be used to quell Nipah virus at the source in fruit bats rather than waiting for infection to spread to humans, which will expel the epidemic risk that Nipah currently poses.

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Abstract 1255

Synthesis of Known Smoothened Inhibitor Azologues

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Craig Streu

My research focuses on developing light-responsive versions of known smoothened inhibitors. Basal cell carcinoma, lung, breast, brain, prostate, and many more cancers can be caused by unregulated activation of the Hedgehog (Hh) signaling pathway. When Patched-1 is mutated and cannot properly repress Smoothened (Smo), Smo continually activates the protein complex that allows for Hh target genes to be regulated. The constant activation of these genes causes tumorigenesis in many types of tissue. Smoothened inhibitor drugs carry out the job of Patched-1 when a mutation prevents it from performing correctly. Although effective smoothened inhibitors exist, they are often accompanied by severe side effects that result from off-target activity. To more effectively target these drugs to reduce off-target activity an azo moiety has been incorporated into the design of these drugs. These nitrogen-nitrogen double bonds change conformation in response to light, and as such, can change the shape, electronic properties, and bioactivity of the molecules in which they are incorporated. The drugs are designed to be taken in their inactive form and activated by photoisomerization in response to light administered selectively to the cancer cells, thereby leaving healthy cells unharmed. Azologues of smoothened inhibitors in particular represent especially challenging synthetic targets. This work delineates the specific synthetic challenges and outlines the relative success of different synthetic strategies for overcoming these issues on two well-known smoothened inhibitors.

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Abstract 1257

Hydrophilic Chitosan-Nanoparticles for Nanomedicinal Applications

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Chitosan (CS) is a fibrous compound derived from chitin shells of crustaceans such as shrimp, lobsters, and crabs. It is an abundant polysaccharide having all the important factors, such as biodegradability, biocompatibility, hydrophilicity, and non-toxicity, that are necessary for nanomedicinal applications. Chitosan nanoparticles (CSNPs) are used widely in biomedical applications such as contrast agents in medical imaging and for drug and gene delivery into tumors. In this work, we use a hydrophilic chitosan nanoparticle preparation technique based on the ionic gelation process. This is an extremely mild process involving mixtures of two phases at room temperature. One phase contains chitosan and polyanion sodium tripolyphosphate (TPP) and the other contains a diblock copolymer of polyethylene oxide (PEO) and polyglycolic oxide (PPG or PEO-PPG for both). X-Ray diffraction (XRD) and transmission electron microscopy (TEM) was made on the CSNPs to determine their structural characteristics and average size. CSN samples were prepared using 0.5% and 1.0% W/V of CS where the latter were also incorporated with PEO and PEO-PPG. The size of the CSNPs was in the range of 9–51 nm for 0.5% W/V of CS with a zeta potential of ~13 mV, 12–65 nm for 1.0% W/V concentration of CS with a zeta potential ~6 mV, 7–24 nm for 1.0% W/V concentration of CS incorporated with PEO having a zeta potential ~9 mV and 19–49 nm for 1.0%W/V concentration of CS incorporated with PEO-PPG having a zeta potential of ~21 mV. Our results show that the size and the aggregation of nanoparticles can be conveniently modulated by varying the ratio of CS/PEO-PPG and CS/TPP. XRD and TEM analysis confirms the semi-crystalline structure of the CSNPs with the 0.5% W/V concentration of CS sample showing considerably greater degree of crystallinity than the 1.0% W/V concentration of CS sample. However, incorporating the PEO-PPG results in a significant increase in the crystallinity of the CSNPs. Future work will include measurement of the efficacy of drug delivery using our CSNPs in cancer culture studies.

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Abstract 1258

Synthesis and evaluation of imidazole-bearing chalcones as a potential treatment for pulmonary aspergillosis

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Bradley Ashburn, Helmut Kae

Pulmonary aspergillosis (PA) is a category of respiratory illnesses that significantly impacts the lives of immunocompromised individuals. However, new classifications of secondary infections like influenza associated aspergillosis (IAA) and COVID-19 associated pulmonary aspergillosis (CAPA) only exacerbate matters by expanding the demographic beyond the immunocompromised. Meanwhile anti-fungal resistant strains of Aspergillus are causing current treatments to act less effectively. Symptoms can range from mild (difficulty breathing, and expectoration of blood) to severe (multi organ failure, and neurological disease). Millions are affected yearly, and mortality rates range from 20–90% making it imperative to develop novel medicines to curtail this evolving group of diseases. Chalcones and imidazoles are current antifungal pharmacophores used to treat PA. Chalcones are a group of plant-derived flavonoids that have a variety of pharmacological effects, such as, antibacterial, anticancer, antimicrobial, and anti-inflammatory activities. Imidazoles are another class of drug that possess antibacterial, antiprotozoal, and anthelmintic activities. The increase in antifungal resistant Aspergillus and Candida species make it imperative for us to synthesize novel pharmacophores for therapeutic use. Our objective was to synthesize a chalcone and imidazole into a single pharmacophore and to evaluate its effectiveness against three different fungi from the Aspergillus or Candida species. The chalcones were synthesized via the Claisen-Schmidt aldol condensation of 4-(1H-Imizadol-1-yl) benzaldehyde with various substituted acetophenones using aqueous sodium hydroxide in methanol. The anti-fungal activity of the synthesized chalcones were evaluated via a well-diffusion assay against *Aspergillus fumigatus*, *Aspergillus niger*, and *Candida albicans*. The data obtained suggests that chalcone derivatives with electron-withdrawing substituents are moderately effective against Aspergillus and has the potential for further optimization as a treatment for pulmonary aspergillosis.

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Abstract 1272

Progress Toward the Synthesis of a Next-Generation Tyrosine Kinase Inhibitor

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Mariah Brenz, Theodore Hirschfield, Craig Streu

There are currently hundreds of drugs that are FDA approved to treat cancer and many fold that number in various stages of development. The function of these chemotherapy drugs is to wipe out dysfunctionally proliferating cancer cells. The drawback of these drugs is that they not only target cancer cells, but can also harm healthy cells along the way, which contributes to the side effects commonly associated with these chemotherapies. One such drug specifically for chronic myeloid leukemia works by inhibiting multiple tyrosine kinase proteins. Herein we disclose the design, and progress toward the synthesis, of a photoswitchable version of a chronic myeloid leukemia drug with the goal of eliminating its off-target side effects. This can be done by integrating an azo bond into the architecture of the known drug. Such azologues are able to undergo light-induced isomerization. It is possible to exploit this shape change such that the drug can be reversibly activated with light. The chief advantage of these drugs is that they can be activated selectively in the afflicted tissue without causing major side effects throughout the rest of the body. Herein we describe progress toward the synthesis of a photoswitchable azologue of a validated tyrosine kinase inhibitor for treating chronic myeloid leukemia.

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Abstract 1273

Synthesis and Biological Evaluation of a Novel Photoswitchable Immune Checkpoint Inhibitor

Diana Kernen, *Albion College*

Craig Streu

Checkpoint inhibitors are among the most important recent developments in cancer therapy. These checkpoints are critical interactions between immune receptors and tumor cells, which prevent the immune system from recognizing and destroying tumor cells. Checkpoint inhibitors disrupt these interactions, allowing the immune system to recognize and destroy tumor cells. However, by interfering with the immune system's self versus nonself recognition system these drugs can result in a broad array of side effects such as joint pain, confusion, seizures, headaches, and chest pain. Additionally, such checkpoint inhibitors are almost exclusively monoclonal antibodies, which are expensive to produce and require intravenous administration, generally in a medical setting. To address this issue, small molecule checkpoint inhibitors have been developed that lower production costs, increase stability, improve tumor penetration, and can be given orally. These molecules have surfaced on the market, but they too result in side effects for patients. However, it may be possible to avoid these potentially dangerous side effects by selectively activating the immune system specifically at the site of the solid tumor, so as to prevent the off-targeting autoimmune responses and reduce the potential for undesired side effects. Herein, we disclose the synthesis and testing of a novel photopharmaceutical and progress toward the synthesis of a second generation derivative that may be used as a site-selective checkpoint inhibitor.

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Abstract 1274

Progress Toward the Synthesis of a Photoswitchable Kinase Inhibitor

Noah Rollison, *Albion College*

Noah Rollison, Diana Kernen, Sathwik Reddy, Craig Streu

Chemotherapy drugs that target aspects of cell division and growth have been prescribed to cancer patients for decades. Often with these life saving treatments, patients experience life-altering side effects because the drugs not only target cancerous cells, but healthy ones as well. As such, improvements in drug targeting are among the most promising developments in cancer treatment. A particularly exciting method of targeting drugs is with light, which is known as photopharmacology. Azo compounds, which contain a nitrogen-nitrogen double bond that can be reversibly isomerized between cis and trans forms in response to visible wavelengths of light, are among the most common types of photopharmaceuticals given their general stability and highly reversible photodynamics. Since drugs bind to their targets as a result of shape and charge complementarity, the ability to change conformations in response to light allows the drugs to change from a deactivated 'off' form, to an active 'on' form. The drug's ability to be selectively switched on and off allows it to be administered in a deactivated state and activated selectively at the site of the tumor with specific wavelengths of light. As a result, photopharmaceuticals have the potential to prevent patients from experiencing the adverse side effects that result from off-target interactions of traditional chemotherapy drugs. Herein we describe the design and synthesis, and unique synthetic challenges for a novel azologue of a commercial kinase inhibitor.

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Abstract 1276

ChlorOFF: Discovery, Directed Evolution, and Cellular Applications of Fluorescent Protein-based Sensors for Chloride

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Chloride is the most abundant biological anion, and an increasing body of evidence points to its essential roles in normal physiology and disease. This understanding has been significantly aided by genetically encoded fluorescent sensors. To date, these have primarily been engineered from the green fluorescent protein (GFP) found in the jellyfish Aequorea victoria. However, to study chloride in diverse biological contexts, new sensor scaffolds with unique properties must be developed. To achieve this, we first explored the rich sequence space of the GFP family using a stepwise bioinformatics approach and discovered starting points found in organisms from diverse marine niches. Following this, directed evolution methods were developed to improve the sensor properties, resulting in ChlorOFF – a high-affinity and turn-off sensor for chloride. Through this approach, we have not only identified new mutational hotspots that contribute to anion sensitivity in the GFP family but also revealed how cells mobilize chloride in a cystic fibrosis model.

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Abstract 1289

Multiplexed illumination of RNAs in live mammalian cells by fluorescence lifetime imaging microscopy

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Nadia Sarfraz

RNA functions are closely linked to their subcellular localizations. Acute cellular perturbations and disease mutations affect subcellular RNA dynamics and hence their functions. Visualizing RNAs via fluorescence microscopy using robust fluorescent RNA tagging and tracking approaches is an active area of research and of critical importance to understand RNA functions in the complex cellular environment. Knowing when and where RNA localizes over time, relative to other cellular molecules and in the context of cellular perturbations, will allow detailed insights in mechanistic underpinnings of RNA functions. Techniques to achieve quantitative visualization of RNAs in live cells over time are an active area of research. What's more, robust approaches to label and track multiple RNAs simultaneously live are entirely absent. Here, we explore fluorescence lifetime imaging microscopy (FLIM) as a novel approach to design a fluorescence RNA sensor with entirely new and orthogonal imaging capabilities. Our FLIM RNA imaging platform is based on a short RNA tag that binds a small organic molecule with fluorescence properties. Tight binding to the RNA changes fluorescence lifetime of the fluorescence probe, yielding strong intensity-independent contrast in live cells. We systematically altered the RNAs of interest that we genetically fused with our FLIM sensor, and investigated RNA imaging in various cell systems. We found that the fluorescence tag performs independent of the RNA it is genetically tagged to, and independent of the cell type, confirming robustness of the imaging platform. The RNA tag is derived from a bacterial riboswitch that consists of large phylogenetic diversity. We found that changing the RNA tag sequence by exploring this phylogenetic diversity may maintain affinity to our probe but alters fluorescence lifetime. We systematically assessed fluorescence lifetime contrast live for a series of different RNA sensor sequences as the basis for visualizing multiple RNAs in the same cell simultaneously. We demonstrated multiplexing capabilities by quantitatively imaging multiple RNAs with different localization patterns in the same cell. Together, our RNA FLIM platform allows robust visualization of complex RNA subcellular localization dynamics with the potential of broad applicability of RNA biology investigations across fields. Current efforts are underway to expand multiplexing capabilities to add more orthogonal RNA tags and to explore imaging RNAs in complex cell systems.

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Abstract 1291

Synthesis of Hemithioindigo Compounds as Microtubule Polymerization Inhibitors

Alexis Moss, *Albion College*

Noelle Robert, Vedha Reddy, Clara Wagner, San Pham, Craig Streu

Regulation of cell growth is critical to health as uncontrolled cellular division can result in a host of medical pathologies including cancer. One essential step in mitotic cell division is the formation of microtubules, in which allow the two copies of DNA to be separated and continue cell division. This is known to be a target in cancer therapies, however, with mitotic cellular division occurring everywhere, cancer therapies result in having off-target activity, creating significant side effects. A solution to this problem is selectivity. Nowadays there are modern technologies that selectively choose the intended target, such as, molecular photo switches. Synthesizing compounds that can change shape based on which wavelengths of light are being absorbed giving said compounds an on and off switch. In doing so, introduces a drugs ability to reversibly switch shapes in response to light. Using rational drug design, improvement on the bioactivity and photoswitchability of these inhibitors was synthesized and tested. With the help of computational programming, modeled with colchicine protein structures, complementary binding sites have been identified and targeted. Redesigning of the photoswitchable drug synthesis was done to conduct Suzuki reactions to complement the adjacent compounds at the binding sites of the colchicine protein structures. Improving on the binding affinity of the binding sites, ultimately improves the potency and efficacy of the photoswitchable inhibitor. This synthesis outlines redesigning and improving off target activity of cancer therapies commonly used today.

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Abstract 1293

California yerba santa, Eriodictyon californicum, as a novel protein tyrosine phosphatase 1B (PTP1B) inhibitor

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Jasmine Rodriguez, Julia Starczewski, Mark Vincent dela Cerna, Savita Chaurasia

Protein tyrosine phosphatase 1B (PTP1B) is the founding and archetypal member of the protein tyrosine phosphatase (PTP) superfamily, the largest phosphatase family encoded by the human genome. PTPs, such as PTP1B, regulate cellular phosphorylation levels in conjunction with protein tyrosine kinases (PTK). Aberrant cellular phosphorylation is a commonality in many diseased states. PTP1B was initially studied as a therapeutic target in type 2 diabetes and obesity due to its role in the attenuation of the insulin and leptin signaling pathways. Furthermore, its activity has been suggested to play a role in the regulation of anti-apoptotic pathways, pro-metastatic signaling, and cell-to-cell adhesion. Recent studies have elucidated the pro-oncogenic and tumor-promoting activities of PTP1B in several cancers including pancreatic, breast, and ovarian cancers, among others. As such, PTP1B presents as a promising drug target. However, the discovery of potent and selective PTP1B inhibitors has proved challenging; despite being the most well-studied PTP with more than 750 known inhibitors, there are currently no FDA-approved drugs that act on PTP1B. This study implicates Eriodictyon californicum (commonly known as California yerba santa, santa herba, and holy weed) extract as a novel inhibitor for PTP1B. Yerba santa is a plant native to North America, traditionally used by Native Americans, primarily due to its antioxidant and anti-inflammatory properties to treat conditions such as asthma. Yerba santa is designated safe for human consumption by the FDA and has recently demonstrated promising effects against Alzheimer's, obesity, and some cancers. In this present study, phosphatase inhibition assays revealed that ethanolic yerba santa leaf extracts inhibited PTP1B in a dose-dependent manner with an IC50 of 4 μg/ml. Sodium orthovanadate was used as a positive control. NMR and molecular docking were also used to confirm if any molecules present in the extract bind to PTP1B. Natural products have long been a source of scaffolds for drug design, and it's our hope that this study will lead to discovery of potent and selective PTP1B inhibitors. Finally, this study also begins to uncover the cellular mechanisms of the preventative effects of this highly important but relatively understudied plant.

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Abstract 1296

Progress toward the synthesis of photoswitchable prosthetics for common antibiotics

Paul Volesky, *Albion College*

Noah Rollison, Craig Streu

Bacterial resistance has been an emerging public health issue since the discovery of penicillin in the1940s. Today, overprescription and agricultural uses have accelerated the development of resistance to new antibiotics. Soon, our ability to treat bacterial infections or even perform common surgical procedures could be seriously compromised by the proliferation of resistant strains. At the same time, research and development into novel antibiotics has slowed to a pace not seen since the first commercialization of penicillin. One obstacle to commercialization of new antibiotics is the development of molecules that are safe for eukaryotic cells while maintaining high toxicity for bacteria. New strategies must be developed to overcome this obstacle. One such solution is the development of compounds that can be selectively activated in the infected tissue using light. Use of these compounds, known as photopharmaceuticals, can prevent damage to the surrounding tissue. One common approach for the development of photopharmaceuticals is the incorporation of a photo-isomerizable prosthetic group that changes the shape, and therefore the activity, of a drug in response to light. This project aims to incorporate such a prosthetic group into the structure of a well-known antibiotic for spatial control of antibiotic activation. We herein outline the rational design and the progress toward the synthesis of two subtypes of photo-sensitive antibiotics.

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Abstract 1298

Design of a novel optogenetic tool for electroneutral elevation of intracellular pH

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An emerging hallmark of cancer is alkalization of the cytoplasm and acidification of the extracellular matrix. This reversal of the pH gradient has been shown to enhance cancer cell behaviors, but little is known about the underlying mechanism or molecular drivers. Optogenetic tools to manipulate pH offer unique advantages for revealing the mechanistic role of pH in cancer cell behaviors by enabling reversible manipulation of intracellular pH (pHi) in a single cell. We recently developed an optogenetic tool to spatiotemporally raise pHi based on light-activated proton pumps. However, this tool is electrogenic (alters membrane potential as well as pHi) and is not ideal for long-term pHi manipulation (hours to days). Here, we have created first-generation optogenetic tools capable of raising pHi without altering membrane potential and that are compatible with long-term pHi manipulation. Our tools are based on the sodium-proton exchanger, NHE1, which is an electroneutral pH homeostatic regulatory protein. NHE1 activity is inhibited by allosteric sequestration of the C-terminal tail away from the plasma membrane. We engineered a small library of NHE1 fusions to light-sensitive binding partners, LOV2 and Zdk1 (Opto-NHE1 library). In the dark, LOV2 and Zdk1 interact to sequester the C-terminal tail of NHE1 from the plasma membrane keeping NHE1 inhibited. Light activation releases this inhibition and activates NHE1. We show that several Opto-NHE1 variants from this library exhibit robust light-sensitive pHi increases using both population-based assays and single-cell confocal microscopy experiments. We next determined that expression of the tools alone do not alter pHi, it is only upon light activation that pHi is altered. Importantly, the increased pHi induced by Opto-NHE1 is similar to that observed during transformation (0.2–0.5 pH units). We can apply Opto-NHE1 to specifically study the role pHi plays in cancer initiation, progression, and metastasis. Furthermore, the protein engineering approach described here is adaptable to the generation of other light-activatable ion transporters. Future work will apply this approach to generate a panel of light-sensitive electroneutral tools that can raise and lower pHi through light-dependent transport of protons, bicarbonate, and other ions.

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ASBMB

Abstract 1304

Synthesis and analysis of a novel light-activated bcr-abl inhibitor

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Chemotherapeutics have been very effective in the treatment of various cancers. However, off-target binding of these drugs often results in a litany of side effects. One way to combat this issue is to make drugs that are very specific to the binding site of their intended target. Such specific binders, although challenging to achieve, may limit many side effects, but other issues like tissue selectivity generally still remain. One way to improve tissue selectivity is to develop photopharmaceuticals. Photopharmaceuticals are a class of drugs that can be activated and deactivated in response to light. Among the most common methods of photopharmaceutical design is the incorporation of an azo bond into an established pharmaceutical compound. This azo group is able to reversibly isomerize in response to light, which changes the shape, and ultimately the binding affinity of the drug fro its target. We herein outline the design, synthesis, and unique synthetic challenges associated with the development of a photopharmaceutical for the treatment of chronic myeloid leukemia (CML).

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Abstract 1321

Fungal Cell Wall Structure and Remodeling by Antifungal Drugs Elucidated Using Solid-State NMR Spectroscopy

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Invasive fungal infection has high occurrence and mortality among immunocompromised patients. Most of the currently available antifungal agents have limited efficacy, relatively high toxicity, and emerging resistance. The carbohydrate-rich cell wall is a promising target for antifungal drugs, but we lack in-depth knowledge of the structure, assembly, and dynamics of cell wall biomolecules. To overcome this barrier, we have explored the use of solid-state NMR (ssNMR), a nondestructive and atomic-resolution technique, for characterizing the polysaccharides and proteins in intact and living fungal cells. The method allowed us to understand the structural remodeling of fungal cell walls in response to mutations, antifungal drugs, and different morphological stages of the life cycle. First, we combined ssNMR, functional genomics, and biochemical analysis to identify the functionality and diversity of cell wall carbohydrates in *Aspergillus fumigatus* and four mutants depleted of major structural polysaccharides. We revealed a rigid inner core of the cell wall formed by tightly associated chitin and α-1,3-glucan, which are embedded in a soft matrix of β-glucans and capped by a mobile outer shell rich in galactosaminogalactan and galactomannan. The distribution of α-1,3-glucan in chemically and dynamically distinct domains supports its diverse functions in structure and pathogenicity. Second, we characterized the structural responses of *A. fumigatus* cell walls to the treatment of the caspofungin drug. The drug-treated cell walls are highly hydrophobic and stiff to resist external stress. The fungi also increased the content of chitin and produced new forms of α-glucans to compensate for the loss of α-glucan due to drug inhibition. Third, we found that the crystalline structure of chitin bears intrinsic heterogeneity, which is resistant to caspofungin treatment. We documented the structural fingerprints of chitin across *A. fumigatus*, *Aspergillus sydowii*, *Aspergillus nidulans*, *Rhizopus delemar*, *Candida albicans* and *Candida auris*. Finally, we characterized the cell wall organization in different morphotypes that varied along with the life cycle and growth conditions, revealing a conserved carbohydrate core in conidia and mycelia. These studies and the biophysical technique yield essential information about carbohydrate components and structures of the cell walls and their packing interfaces at atomic levels that can serve as potential targets for discovering novel antifungal compounds with broad spectrums and improved efficacy.

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Abstract 1335

Natural history of biomarker changes associated with gut inflammatory disease in postnatal human development

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Necrotizing enterocolitis (NEC) is a dangerous medical emergency in preterm infants associated with aberrant communication between gut microbiota and their human host. Severity of NEC varies widely between individual infants. Clinical decision making relies heavily on x-ray, physical symptoms, and lab findings from blood draws; these tests only detect advanced disease stages with high mortality and comorbidity rates. They also are used as monitoring biomarkers, but lack sensitivity, specificity, and positive predictive value. Here, we compare the natural history of current biomarker changes with fecal intestinal alkaline phosphatase (iAP) during NEC disease progression in a four-hospital population of 269 preterm infants; 25% had mild to severe NEC and 75% did not develop NEC. Continuous monitoring of preterm infants demonstrated that biochemical changes in iAP could be observed before x-ray diagnosis, as well as during clinical management. There is a predictable order of iAP changes for NEC in its early stages, making it suitable for preventive treatments. One week prior to x-ray or clinical confirmation of NEC, a five-fold elevation of iAP protein abundance and a two-fold decrease in iAP biochemical activity was observed compared to post-conceptional age-matched controls. Post-clinical diagnosis, current management practices for NEC highlight that iAP biomarker changes that are associated with divergent disease trajectories.Surveillance of severe NEC infants showed sustained iAP biochemical dysfunction and persistent increase in iAP abundance 3 weeks after diagnosis which was not observed in suspected NEC and non-NEC infants. Our data suggest that continuous monitoring of iAP abundance and biochemical activity could allow for earlier disease diagnosis that may help direct management and reduce the risk of NEC recurrence.

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ASBMB

Abstract 1352

SAXS for NANPs: Advancing the structural characterization of nucleic acid nanoparticles through small-angle x-ray scattering

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RNA has significant therapeutic and clinical potential, as evidenced by the success of three siRNA drugs and two messenger RNA vaccines. Nucleic acid nanoparticles, NANPs, allow for combinations of multiple functional moieties (i.e. siRNAs, fluorescent tags, aptamers, antisense oligos, etc…) to be delivered to cells in precise stoichiometric ratios. One of the current limitations for the further application of NANPs lies in the accurate assessment of their structure, as it has been found the structure and level of functionalization significantly impacts their recognition by the immune system. In order to more fully understand this phenomenon, a more thorough structural characterization of NANPs is required. Traditional methods of structure confirmation for NANPs involves a combination of atomic force microscopy, electrophoretic mobility shift assays, and dynamic light scattering. While this combination is useful for confirming the general structure and homogeneity of NANPS, it does not provide many quantitative details about the native 3D structure of the NANP, as each technique has some significant limitations. Using a hexameric RNA nanoring able to carry up to six dicer-substrate RNAs, we have shown that the addition of small-angle x-ray scattering to this combination of characterization techniques allows for increased quantitative and qualitative information to be discerned and for the 3D structure to be accurately reconstructed.

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Abstract 1359

Extraction and Characterization of Black Seeds (*Nigella sativa*) Proteins by ESI QTOF LC/MS/MS

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Medicinal plants are an enormously rich source of biologically active phytochemicals such as sterol, fatty acid, alkaloids, and other phenolics. These compounds extracted from various plant components, such as fruit, seeds, leaves, flowers, etc., play a crucial role in treating several ailments. However, limited studies have been reported on the characterization of biologically active proteins and peptides. There are ample opportunities to explore complete proteome analysis utilizing traditional protein purification techniques coupled with high-resolution mass spectrometry. Black seed (*Nigella sativa*), a miraculous medicinal herb commonly known as black seed or Kalonji, belongs to the plant family Ranunculaceae. It has shown promising effects as an anti-inflammatory, promoting weight loss, and lowering blood pressure and cholesterol. Our group has previously reported the anticancer activity of selected proteins extracted from its seeds against the MCF7 breast cancer cell line. The present study focuses on the top-down proteomics evaluation, employing size exclusion chromatography, high-resolution ESI QTOF LC/MS/MS, and De Novo protein sequencing bioinformatics tools to evaluate the Black seeds proteome. The black seeds were ground and defatted in hexane. Proteins were extracted in PBS and recovered by precipitating in 80% ammonium sulfate. The crude protein extract was separated by gel filtration chromatography employing the Superdex-75 column using an automated FPLC system. The crude protein and separated chromatography fractions were analyzed by 10% Tris/tricine SDS-PAGE gel electrophoresis. Reversed-Phase High-Performance Chromatography (RP-HPLC) further analyzed the pooled gel filtration fractions. The performic acid oxidation method was employed for protein modification and was followed by trypsin digestion. The tryptic digests were investigated by high-resolution mass spectrometry establishing the identity of proteins by QTOF LC/MS/MS and data analysis by the De Novo sequencing bioinformatic tool PeakStudio-X. The gel filtration chromatography resolved into four peaks and was further purified by the RP-HPLC. The differential mass spectrometric analysis of tryptic digest revealed exciting information on black seeds proteome. The bioinformatics analysis of tryptic peptides using the SwissProt Viridiplantae database with FDR $\leq$ 1% revealed 81 proteins. The crude contained 54 proteins, while the four gel filtration peaks P1, P2, P3, and P4 contained 57, 59, 13, and 4 proteins, respectively. The number of unique proteins identified in crude is 24, while the gel filtration peaks P1 and P2 identified 12 and 25 unique proteins, respectively. In contrast, no unique proteins were placed in the peaks P3 and P4.

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Abstract 1364

Probing the molecular mechanism of radical enzymes involved in hydrocarbon activation

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Anaerobic microbes are able to degrade hydrocarbons that compose crude oil in the absence of molecular oxygen. These microbes initially activate their relatively inert substrates for downstream metabolism by addition of the alkene fumarate to the hydrocarbon through a hydroalkylation mechanism. In other words, a hydrocarbon C–H bond is homolytically cleaved, the hydrocarbon radical is added to one side of the alkene, and the hydrogen atom is added to the other side of the alkene. This type of reaction is a synthetically attractive method for forming C–C bonds, as it can set up to three contiguous stereocenters without byproducts or pre-functionalized substrates. This chemistry is enabled by a class within the large glycyl radical enzyme superfamily known as X-succinate synthases (XSSs). The glycyl radical cofactor within XSS enzymes initiates this challenging reaction but, up until now, has not been installed *in vitro*. Here, we show how genome mining was used to obtain soluble enzymes capable of installing the glycyl radical cofactor. With these enzymes in hand, we are able to visualize the glycyl radical by EPR spectroscopy and use the activated XSS to catalyze hydroalkylation reactions. These results set the stage to both probe the mechanism of these fascinating catalysts to gain fundamental understanding as well as explore their use as synthetically useful, selective C–H functionalization biocatalysts.

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Abstract 1381

Effects of Piñon Pine Sap and the Component Limonene on Cell Proliferation

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Piñon pine sap has long been used as a traditional Native American healing salve, said to be effective in treating a wide range of skin ailments including eczema, burns, and ulcers. This study investigates a possible connection between facilitated cell proliferation and treatment with piñon pine sap in cultured cells. Both whole sap and components of interest were examined for their ability to increase wound closure rates. Sap analysis by gas chromatography-mass spectrometry identified the cyclic monoterpene, limonene, as a candidate for the chemical compound involved in increased rates of cell proliferation. Subsequent *in vitro* wound healing assays focused on testing D-limonene and whole sap using a melanoma cell line. Wound closure measurements were taken at 0-, 24-, 48-, and 72-hours posttreatment for each assay. Total wound closure percentages were compared across the different piñon pine sap and limonene treatments at each of the measured time intervals. In all wound treatments piñon pine sap had greater total percent closures than those of the controls. The difference in overall percent wound closure rates over the designated time period was an approximate 7.8+4.5% increase in closure time in sap-treated samples compared to cells with no treatment. The D-limonene treatment yielded increased wound closure rates of over 8.3+6.7% in D-limonene treated samples compared to nontreated samples. Treatment with piñon pine sap led to increased rates of cell proliferation. This verifies the long-held traditional use of piñon pine sap as a wound healing ointment.

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ASBMB

Abstract 1389

Picoliter Thin Layer Chromatography (PicoTLC) to Assay Lipid Signaling in Single Cells

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Introduction: Thin layer chromatography (TLC) is a widely used analytical technique for a many applications, but its use for extremely miniature samples, such as a single cell with a volume of ~1 picoliter, has not been realized to date. A major challenge is that the dimensions of adsorbent layer in conventional TLC significantly exceeds that of single cells. To address this challenge, we have developed a new microfabricated TLC platform by creating an array of microchannels filled with monolithic porous silica. The channels reduce the lateral diffusion and confine the movement of compounds along the microchannels, enabling sample separation and detection of samples of the picoliter volumes. The platform is termed picoliter TLC (PicoTLC).

Materials and Methods: The PicoTLC microchip was fabricated by combining sol-gel chemistry with microfabrication. PicoTLC incorporates an array of microscale channels made from a highly porous monolithic silica. The channels were designed to accept picoliter-scale volume samples and possessed a width and depth for each channel of 80 μm and 13 μm, respectively. Droplets of fluorescent compounds with a volume of ~9 picoliter were spotted on the microchannels and then separated upon exposure of the channel inlet to organic solvents. To demonstrate single-cell analysis, single K562 cells loaded with sphingosine-CY5 were spotted on the microchannels. The cellular metabolites of sphingosine-CY5 were extracted from the individual cells and separated using PicoTLC.

Results and Discussion: To demonstrate the utility of PicoTLC, three groups of model lipid compounds, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(carboxyfluorescein) (18:1 PE CF) and 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (Texas Red DHPE), BODIPY-FL conjugated phosphatidylinositol-4,5-bisphosphate (PIP2) and phosphatidylinositol-3,4,5-triphosphate (PIP3), and fluorescein conjugated sphingosine (Sph) and sphingosine 1-phosphate (S1P) were assessed. The lipids were dissolved in ethanol and spotted at the inlet of the channels of a PicoTLC chip, followed by separation. The results demonstrated that PicoTLC can separate picoliter samples. S1P is a metabolic product of Sph formed by the action of sphingosine kinase (SK) as part of the sphingolipid signal transduction pathway within cells. To further demonstrate the power of PicoTLC, single cells were assayed for SK activity. Single cells loaded with Sph-CY5 were spotted on a PicoTLC microchannel. The PicoTLC chip was developed and the fluorescence of CY5 measured within the channel. Two fluorescent analytes (sphingosine-CY5 and sphingosine-1-phosphate-CY5) were extracted from the cell, and separated along the microchannels in 4 min (resolution = 1.8). To confirm that the conversion of Sp-CY5 to S1P-CY5 was due to SK, cells were analyzed after incubation with the SK inhibitor PF543. As expected, incubation of cells with the inhibitor abrogated the S1P-CY5 peak. Analysis of 19 single cells (±PF543) demonstrated the heterogeneity of SK activity at the single cell level as well as the inhibition of SK (p-value $\leq$0.0001). This result demonstrated that PicoTLC could be used for analyzing biologically reactions inside single cells.

Conclusion: We have developed a TLC technology for separating the components of picoliter-scale specimens including single cells. Further PicoTLC was demonstrated for the assay of sphingolipid signaling activity in single cells. We envision PicoTLC will have potential applications in many areas where miniature specimens and high-throughput parallel analyses are needed.

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Reference

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Abstract 1400

On-chip Pumping Conditions for Single Cell Measurements

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We are developing a microfluidic system with high temporal resolution cell stimulation for high-throughput single-cell measurements. Our research goal is to measure how cells respond to oxidative stress and nutrient deprivation by developing molecular and microfluidic tools for single cell analysis by chemical cytometry. Chemical cytometry is a technique in which individual cells are lysed and their contents are separated electrophoretically for analysis. Previous applications of microfluidic chemical cytometry have involved stimulating the cells off the microfluidic chip prior to measurement. This process causes variable delay between cell stimulation and observation, which may affect results. Thus, we are designing an on-chip pumping system so that cells interact with the stimulus for a defined period and then transit the device to the lysis point and analysis channel. These procedures will allow us to measure cell signaling in the social amoeba *Dictyostelium discoideum* as a model of non-genetic cellular heterogeneity. *D. discoideum* uses cAMP as an extracellular signal to direct cell movement into social development. Within *D. discoideum's* unique social cycle, we are specifically analyzing protein kinase B (PKB) activity when the amoebic cells become aggregation competent. Activated PKB carries signals to initiate chemotaxis, and in the single cell stages of development its activity can vary significantly. We have used fluorescent microbeads to characterize pressure-driven and electrokinetic flow in the microchannels. Currently, we are introducing *D. discoideum* cells onto the device and investigating the effect of stimulus duration on cell response to reactive oxygen species to demonstrate feasibility.

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Abstract 1401

Diptoindonesin G is a middle domain HSP90 modulator for cancer treatment

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Our lab previously identified a natural compound diptoindonesin G (Dip G) that promotes estrogen receptor alpha (ER) degradation. The objective of the study is to determine the mechanism of action of Dip G in human breast cancer cells. Dip G binding to HSP90 was measured by fluorescence polarization and estrogen receptor (ER) degradation was shown by proteomics and western blotting. The effect of Dip G on ER transcriptional activity was measured by RNA-seqencing. Our results showed that Dip G is an HSP90 modulator by binding to the middle domain of HSP90 (Kd = 0.13 uM) without inducing heat shock response. Binding of Dip G to HSP90 promoted degradation of HSP90 client proteins including ER, a major oncogenic driver protein in most human breast cancers. Because Dip G does not directly bind to the ligand binding domain (LBD) of ER, Dip G promotes ER degradation regardless of the ER mutation status. ER mutations in the LBD is the major mechanism for endocrine resistance. The current endocrine therapeutic agents bind to LBD, thus they are largely ineffective for ER mutant expressing tumors. Dip G promoted wild type and mutant ER degradation with similar efficacy. Mutant ER degradation was accompanied by inhibition of expression of ER target genes and cell proliferation. Our data suggest that Dip G is not only a molecular probe to study HSP90 biology and the HSP90 conformation cycle, but also a new therapeutic avenue for various cancers, particularly endocrine-resistant breast cancer harboring ER LBD mutations.

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ASBMB

Abstract 1416

Optimizing the Co-Expression and Purification of Human Circadian Protein Complex CLOCK/BMAL1

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The circadian rhythm is the internal clock in the body that cycles in approximately 24 hours. It controls many physiological changes such as the sleep-wake cycle and body-temperature fluctuation. In humans, every single cell has its own circadian rhythm that is synchronized by a master clock in the brain. At the molecular level, circadian rhythms of individual cells are controlled by the transcriptional-translational feedback loop (TTFL). This loop is initialized by the heterodimerization of circadian locomotor outputs cycle kaput (CLOCK) and brain muscle ARNT-like 1 (BMAL1). The long-term goal of the project is to study the structure and the function of the full-length human CLOCK/BMAL1 complex. Human CLOCK and BMAL1 genes have been cloned into a bacterial expression system. In order to reach the long-term goal, it is necessary to optimize the conditions of expression and purification of the CLOCK/BMAL1 complex to a high quality and homogeneity. In this study, cell density, concentration of the inducer, induction time, and cell lysis methods have been optimized through multiple series of expression. Protein purification trials have been performed to seek the best protocol in order to achieve high yield and good homogeneity. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western Blotting has been used to assess the quantity and quality of the CLOCK/BMAL1 expression. Expression and purification of the CLOCK/BMAL1 proteins will build a solid foundation for their structural and functional studies of the complex. This will deepen our understanding of the molecular mechanism of the circadian rhythm facilitating the design of therapeutic reagents to treat circadian related diseases such as sleep-wake disorder and shift work disorder.

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Abstract 1425

Regulating hypoxia response with small molecules: NMR and X-ray crystallography reveal binding mechanisms of two ARNT-TACC3 disruptors

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The hypoxia inducible factors (HIFs) are heterodimeric transcription factors that play crucial roles in cellular response to hypoxic stress. Dysregulations of the HIF pathway have been associated with various diseases such as cardiovascular and angiogenic remodeling, ischemia, and cancers. Consequently, therapeutic strategies designed to modulate HIF activities have been developed, but none of which effectively target all HIF isoforms. One novel approach is to target the interactions between the β-subunit of HIF (HIF-β, also known as ARNT) and HIF coactivators, as some coactivators are recruited to HIF heterodimers via ARNT which is shared among all HIF-α isoforms (HIF-1α, -2α, and -3α). Through an NMR-based ligand screen, we previously identified several ARNT PAS-B domain binding ligands, two of which (KG-548 and KG-655) were further shown to antagonize ARNT binding to the TACC3 coactivator *in vitro*. Here, we report our investigation of the binding mechanisms of both ligands. Combining data from X-ray crystallography and several solution NMR methods ($^{15}N/^{1}H$- and $^{13}C/^{1}H$-HSQC, ^{19}F, and filtered HSQC-NOESY experiments), we identified KG-548 as a surface binder, interacting with side-chain methyl groups of residues I364 and I458 on the surface of the ARNT PAS-B domain. KG-655, on the other hand, exhibited dual binding modes, with one mode occupying the same surface binding site as KG-548, and another within a water-accessible internal cavity of the protein. Both binding sites are of great interest: the internal binding mode is reminiscent of how an existing drug (belzutifan) interacts with the structurally similar HIF-2α PAS-B domain, the surface binding site on the other hand, is also the proposed binding site for both HIF-α subunits and several coactivators, making it an interface 'hotspot' that warrants further investigation. This work provides insights into the binding mechanisms of ARNT-targeted small molecules and lays the groundwork for designing higher affinity ARNT/coactivator inhibitors.

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Abstract 1436

Cinnamaldahyde enhances the inactivation of *Salmonella enterica* by High Voltage Atmospheric Cold Plasma in Raw Pineapple Juice

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Aubrey Mendonca

Developing methods of pathogen reduction has been a challenge in the food industry for years. Common methods such as pasteurization, canning and the use of antimicrobial compounds all have downsides such as product damage and lessened effectiveness due to the development of antimicrobial resistance. Nonthermal technologies have the potential to solve these issues. High Voltage Atmospheric Cold Plasma (HVACP) is a nonthermal technology that utilizes an active gas to kill pathogens. This active gas is created by passing an electric field through atmospheric air which creates reactive oxygen and nitrogen species that are able to cause damage to cell membranes. A possible solution is hurdle technology that uses a combination of cinnamaldehyde and HVACP treatment to eliminate pathogens on a food product. This study aims to show that a combination of HVACP treatment and cinnamaldehyde can be an effective method to inactivate foodborne pathogens in raw pineapple juice. Raw juice was inoculated with *Salmonella enterica*. To one portion of the juice cinnamaldehyde (100 ppm) was added whereas juice without cinnamaldehyde served as control. After inoculation, the samples were subjected to HVACP treatment for 0, 1, 3, 5, and 7 minutes then stored at commercial storage conditions (4°C) for 24 hours. The samples were analyzed for pathogen survivors using selective and non-selective media. Results indicate a decrease in viability as the exposure time to the HVACP treatment increased. The greatest decrease in viability was observed in juice that was treated with a combination of HVACP and cinnamaldehyde (100 ppm). These differences are reflected on both the selective and non-selective agar plates. Further testing should continue to support the feasibility of this combination of treatments as an industrial pathogen reduction option.

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Abstract 1437

Identification of *Plasmodium falciparum* Autophagy-related Protein 8 (PfAtg8) Inhibitors

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The recent rise in antimicrobial resistance against current malaria treatment options necessitates the identification of alternative malaria targets and new antimalarial drugs. *Plasmodium falciparum* autophagy related protein 8 (PfAtg8) has been identified as one such target, though there are few known inhibitors due to the druggability challenges encountered in targeting protein-protein interactions (PPIs). Kinetic target-guided synthesis (kTGS) is a fragment evolution strategy that has been successfully utilized to identify PPI modulators within the literature. This technique allows the target protein to assemble its own inhibitors from a group of complementary reactive fragments via a biorthogonal, irreversible click reaction. Through a distinct kTGS screening campaign with a multicomponent reaction against PfAtg8, we have identified multiple potential PfAtg8 inhibitors. Hits were identified through mass spectrometry and amplification of products from baseline were analyzed as multicomponent and binary mixtures. Additionally, authentic samples of each hit were synthesized for validation studies as well as further investigation into amplification coefficient perturbances. We are currently conducting biophysical assays on identified PfAtg8 hits.

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Abstract 1438

Implementing an inducible synthetic biology tool for regulating alkaloid biosynthesis in *Catharanthus roseus*

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Krystyna K. Traverse, Carolyn W. Lee-Parsons

Catharanthus roseus, commonly known as the Madagascar Periwinkle, produces two anticancer compounds, vinblastine and vincristine. However, the plant produces low levels of these compounds (0.0002 wt%). While the pathway that produces these compounds has been largely elucidated, its regulation is not well understood. By studying the transcription factors (TFs) that regulate the pathway, we can engineer their expression to enhance production. We are developing a synthetic biology tool that will be used to regulate the expression of our target TFs. We are first optimizing the elements that comprise the tool using expression of the firefly luciferase gene in place of our regulator. To do so, we are studying the impact of an inducible promoter system with different terminators on the expression of the firefly luciferase gene. The system includes the promoter 6 × GAL4 and its synthetic transcription factor GVG, which contains a receptor domain responsive to dexamethasone (DEX). The commonly used nos, 35S, pea3A and 35S+NbACT3+Rb7 terminators were tested. To measure expression, *C. roseus* seedlings were transformed with plasmids via Agrobacterium-mediated infiltration and luminescence was measured. We demonstrated that when the firefly luciferase gene was driven by a constitutive promoter, the 35S + NbACT3 + Rb7 terminator had a 19-fold significant increase in expression compared to the nos terminator. We also tested and confirmed the inducible system was operational upon application of DEX. However, we did note significant baseline expression of the inducible firefly reporter construct even without DEX. The next step is to test our promoter and terminator with a desired fluorescent reporter to be used in hairy roots, our ultimate production platform. In summary, we demonstrated that altering the terminator significantly impacts regulator expression levels. We also showed the inducible promoter system functions in the seedling context. Our ultimate goal is to develop a synthetic biology tool that regulates TFs of our pathway, towards enhancing production of anticancer drugs.

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Abstract 1444

Developing Small Molecule Modulators for Mitochondrial Protein Trafficking

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Primary Hyperoxaluria type 1 (PH1), an autosomal recessive metabolic disorder that is caused by the accumulation of glyoxylate in the liver. Typically, glyoxylate is detoxified by the enzyme alanine-glyoxylate aminotransferase (AGT) through conversion to glycine. However, different mutations in AGT result in the accumulation of glyoxylate, which is converted to oxalate. This disorder provides a system for which to investigate the mechanisms of dual-localized mitochondrial proteins and gain a greater understanding of intracellular protein trafficking. With the goal of developing probes for studying mitochondrial protein import in mammalian cells and, long-term, for specific probes to characterize mitochondrial diseases such as primary hyperoxaluria 1 and Parkinson's disease, a typical shortcoming is that probes from yeast screens may not work well in mammalian cells. In addition, identifying probes that may target a specific mammalian mitochondrial precursor would be beneficial for fine-tuned regulation of protein import.

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Abstract 1482

Erythropoietic Evaluation of *Andrographis paniculata* Leaf Extract in Wistar Rats

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The aim of this study was to evaluate the erythropoietic properties of *Andrographis paniculata* (AP) leaf extract in wistar rats. Traditionally, the leaf is used in the treatment of malaria, pyresis, inflammation and anemia. Blood is an important index of physiological and pathological status of man and animal and the normal ranges of these parameters can be altered by so many factors. Erythropoiesis occurs in the myeloid tissues present in the red bone marrow of the femur, humerus, sternum, ribs and some portions of the skull. It is an active process that requires several metabolites needed for the synthesis of Hb which contains globin as the protein and heam as the prosthetic group. The acute toxicity test (LD50) was done using Lorke's method (1983) in wistar rats The erythropoietic parameters evaluated on AP leaf extract administered orally in wistar rats were Red Blood Cells (RBC), Hemoglobin (Hb) and Packed Cell Volume (PCV). Other parameters analyzed were Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV) concentrations. The animals were divided into 6 groups of 10 rats each: Ctrl = Control group, GrpA: (250 mg/kg AP), Grp B: (500 mg/kg AP), GrpC: (fesolate + 250 mg/kg AP), Grp D: (fesolate and 500 mg/kg AP) and GrpE: treated with fesolate only. They were administered with the different doses of the extract for 7 days and after 24 hrs of the last administration, they were sacrificed and their blood taken by cardiac puncture for erythropoietic analysis. Water and feed were given ad libitum. The result showed that the plant is safe as no animal died in the course of the experiment even at a dose of 5000 mg/kg within 24 hrs. The result also indicated that AP caused significant increase ($p < 0.05$) in the erythrocyte counts as confirmed by increased levels of Hb and percentage PCV likewise fesolate, a standard hematinic drug in comparison with the control. All the treated groups and fesolate showed little or no difference in the Mean Corpuscular Volume (MCV) compared with the control. The treated groups showed no significant ($P > 0.05$) difference on the amount of hemoglobin per unit volume (MCHC) in comparison with the control. In the levels of MCH, the amount of hemoglobin per red blood cell of all the treated groups showed a dose dependent increase compared with the control. Groups C and D treated with both AP extract and the standard drug showed a significant ($P < 0.05$) increase compared with the control. From the result, AP was generally observed to increase the hematological parameters. In normal circumstances, local tissue anoxia apparently leads to the formation of a glycoprotein: the erythropoietin which stimulates increased production of RBC. It is very likely that AP leaf extract may contain an erythropoietin-like agent or blood boosting property which is responsible for the increased production of RBC, Hb and PCV. In conclusion, this study indicates a promising role of *Andrographis paniculata* in the prevention, treatment and amelioration of anemia and provides a pharmacological basis for its use in folkloric medicine in Nigeria.

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ASBMB

Abstract 1508

Small Molecule Regulators of microRNAs in Human iPSC-derived Neurons Identified by High-Throughput Screen Coupled High-Throughput Sequencing

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Background. MicroRNAs (miRNAs) are short, single-stranded RNAs that regulate fundamental biological processes by silencing their mRNA targets. miRNAs are dysregulated in many diseases, and their replacement or inhibition can be harnessed as potential therapeutics. However, current strategies to modulate miRNAs through synthetic oligonucleotides or gene delivery are limited by poor tissue distribution and uptake, immunogenicity, and overall poor therapeutic efficacy. We proposed small molecule compounds as an alternative approach as they usually have better tissue and cell penetrance. Furthermore, compounds already approved for clinical use can be repurposed to accelerate the development of miRNA therapeutics. Methods. To identify small molecule regulators of miRNAs implicated in neurologic disorders, we developed a high-throughput screen coupled high-throughput sequencing in human induced pluripotent stem cell (iPSC)-derived neurons. We demonstrated the utility of the screen by validating compounds that upregulated miR-132, a key miRNA that regulates tau homeostasis and is consistently downregulated in Alzheimer's disease and other tauopathies. Results. We obtained the expression profiles of ~450 miRNAs for ~1400 compounds, including many miRNAs known to be enriched in neurons. We successfully validated that several members of the cardiac glycoside family, which are canonical sodium-potassium ATPase inhibitors, consistently upregulated miR-132 at the sub-μM range. The mechanism was through increased transcription of the miR-132 locus and was mimicked by knocking down ATP1A1 and ATP1A3 – the major ATPase isoforms in neurons. Treating human and rat neurons with sub-μM cardiac glycosides potently downregulated total and phosphorylated tau and protected against toxicity by N-methyl-D-aspartate, glutamate, rotenone, and Aβ oligomers. Conclusions. We described a pipeline and resources for discovering small molecule compounds that regulate specific miRNAs for therapeutic purposes. We also identified and validated small molecule compounds that upregulated the neuroprotective miR-132 in neurons.

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Abstract 1510

Synthesis and Analysis of the SARS-CoV-2 ORF7a Accessory Protein Transmembrane Domain and Determination of the Oligomerization State

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Timothy Reichart

The ongoing SARS-CoV-2 pandemic continues to sicken millions worldwide and fundamentally change the way people interact with each other. In order to better characterize the SARS-CoV-2 virus and potentially develop methods of inhibition for further spread of the disease, this research project focused on synthesizing and characterizing the transmembrane region of the accessory protein ORF7a. ORF7a has been implicated in proper viral assembly, leading to the idea that inhibition of this protein could prevent viral copies from being produced and halt the spread of the virus. The goal of this project was to determine the oligomerization state of the protein through a fluorescence assay in order to better understand the quaternary structure of the ORF7a complex and how it folds. The fluorescence assay is performed using three different samples of the synthesized peptide: one labeled with a TAMRA fluorophore, one labeled with a NBD fluorophore, and the last is unlabeled. After determining the oligomerization state of the protein, potential inhibitors could be synthesized and tested for their efficacy at inhibiting the function of the protein. Further applications of these inhibitors on other viruses can be explored due to the highly conserved nature of transmembrane domains across multiple viral families. Synthesis of the protein was done using a Solid Phase Peptide Synthesis (SPPS) technique and multiple batches of all three samples of peptide have been generated. Characterization and purification were done using High Performance Liquid Chromatography (HPLC) as well as Liquid Chromatography Mass Spectrometry (LCMS). Current research focuses on the purification and quantification of purified ORF7a oligopeptide for implementation of the fluorescence assay.

-Hampden-Sydney College Office of Undergraduate Research.

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Abstract 1513

New approaches to study conformational changes of individual molecules in living cells

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Control of cell behavior rests upon an extraordinary remodeling of the cell, using the same parts to accomplish purposes as distinct as apoptosis and proliferation. These transformations rely on differences in the kinetics or subcellular placement of signaling events, a level of regulation that can only be fully understood by studying protein activities within live cells and animals. This talk will focus on the migration of cancer cells, and how they are guided from tumors to the vasculature by aligned collagen fibers. To study low abundance events and to enhance spatial resolution, we will explore techniques to study the conformation of individual molecules in living cells, using generally applicable approaches. Emphasis will be on Rho GTPase pathways and adhesion molecules regulated by mechanical force. Optogenetic analogs and biosensors of vinculin and talin will be used to study mechanical guidance cues in cancer cell migration, and in phagocytosis.

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ASBMB

Abstract 1525

Modification of Coagulation Factor VIII DNA for Gene Therapy in a Hemophilia A Mouse Model

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Hemophilia A is a clotting disease caused by loss function of coagulation factor VIII (FVIII). In clinical, the prophylactic FVIII infusion is used to alleviate bleeding problem. However, treatment costs are expensive, and repeated infusion's adverse effects will cause an immune response. In this research, we established a nonviral gene therapy strategy to treat the hemophilia A mice. We use the dipalmitoylphosphatidylcholine formulated with iron oxide (DPPC-Fe3O4) as a vector to carry the B-domain-deleted (BDD)-FVIII plasmid DNA and deliver it into the hemophilia A mice via tail vein injection to accomplish the gene therapy. Further, because the FVIII size was too large to enter the cell hard, we used the minicircle system to remove the bacteria backbone of the plasmid to diminish the DNA size. Moreover, to sustain the activity of FVIII *in vivo*, we also substituted E1984V to prolong the lifetime of FVIII. The results showed that the binding ability of circular DNA was confirmed to be more stable than that of linear DNA when combined with DPPC-Fe3O4 nanoparticles *in vitro*. An animal experiment, which IACUC approved, showed that after giving the BDD FVIII DNA, the coagulation problem of the recipients was ameliorated in a few weeks, as tested by assessing the activated partial thromboplastin time (aPTT). However, the duration of treatment did not last long, so we used a modified FVIII DNA, which was E1984V, and the size was reduced to reach the long-term therapeutic effect. After delivering the modified FVIII DNA into hemophilia A mice, the coagulation problem of the hemophilia A mice was improved and lasted for a long time. Further, the mice can achieve a long-term therapeutic effect. The results suggested that this novel gene therapy can improve hemostasis disorder in hemophilia A mice and might be a promising approach to treating hemophilia A patients in clinical settings.

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Abstract 1530

Hybrid Fluorescent Protein and SNAP-tag Biosensors for Extracellular ATP

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Purinergic signaling plays a significant role in many biological processes such as immune responses, neuronal communication, and inflammation. Extracellular adenosine triphosphate (ATP) is a key example of a purinergic signal, and there is still a need for optical tools that detect extracellular ATP to fully understand cell-to-cell communication via purinergic signaling. Previously, we successfully developed genetically-encoded fluorescent protein-based ATP sensors to monitor extracellular ATP levels, but these sensors suffer from high background intracellular fluorescence caused by maturation of the fluorescent proteins before the sensors are transported to the cell surface. To decrease intracellular fluorescence, we are developing hybrid sensors in which we replace one of the fluorescent proteins with the SNAP-tag protein. By using SNAP-tag, we can specifically label our hybrid sensors at the cell surface using a cell-impermeable SNAP-Surface substrate. Through this design, we present two differently colored sensor prototypes, with preliminary spectroscopic characterization and cell surface labeling experiments.

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Abstract 1572

Chemigenetic kinase biosensors for multiplexed activity sensing and super-resolution imaging

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Genetically encoded fluorescent biosensors have revolutionized the study of dynamic biochemical processes in living cells. They enable to investigate intertwined signaling pathways both under physiological and pathological conditions. For instance, kinase activity is often regulated through tight spatiotemporal control via compartmentation. Specifically, protein kinase A (PKA) is organized into plasma membrane nanodomains or sequestered in cytoplasmic phase separated bodies. Due to the small size of these compartments, little is known about the PKA activity organization and regulation within them, even though this knowledge would be crucial to untangle intricately regulated PKA signaling. To investigate these signaling compartments with the necessary spatial resolution and under more physiological conditions new biosensors with enhanced capabilities need to be developed. We therefore set out to develop a new class of kinase activity biosensors by taking a chemigenetic approach, which combines a synthetic fluorophore with a self-labeling protein tag, rendering the resulting biosensors brighter and more photostable than fluorescent protein based ones. Sandwiching a circularly permuted version of the self-labeling protein tag HaloTag7 between a phosphoamino acid binding protein and a PKA specific peptide led to first biosensor prototypes. Rational engineering and screening approaches allowed us to significantly improve the dynamic range of the biosensor. The optimized far-red PKA activity biosensor was successfully applied to advanced multiplexing experiments and is being tested for application in functional super-resolution microscopy via stimulated emission depletion (STED) microscopy. These STED-compatible, far-red PKA activity biosensors should serve as powerful tools to study nano-compartmentalized PKA signaling and its cross-regulation with other signaling networks.

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Abstract 1573

Chemical Biology Approaches to Mapping the PRMT1 Interactome

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Protein Arginine Methyltransferase 1 (PRMT1) methylates arginine residues on proteins including histone H4 which subsequently modulates gene expression. Like other proteins, PRMT1 undergoes post-translational modifications (PTMs), such as acetylation or ubiquitylation, as a part of our proteome's complex regulatory mechanisms. PRMT1 has been implicated as a potential therapeutic target for cancer—both triple negative breast cancer and acute myeloid leukemia. The levels of PRMT1 can be regulated by an acetyl degron that exists at the IKxxxIK motif found at sites K228 and K233; however, the interactions between this motif and other proteins and the mechanisms of proteostasis remain elusive. Moreover, other studies speculate both sites are first deacetylated with site K233 being later acetylated, but the PTM editing enzymes remain unknown. Therefore, we designed a study to explore the pathway of PRMT1 degradation and its regulation by post-translational modifications. In our studies of the PRMT1 interactome, we are using genetic code expansion (GCE), a biochemical tool in which non-canonical amino acids are incorporated in a protein. Through incorporation of acetyl lysine into PRMT1 at the IKxxxIK motif, our work will determine whether acetylation at K233 marks PRMT1 for subsequent degradation by the ubiquitin proteasome pathway. Furthermore, we can incorporate photo-crosslinking groups into PRMT1 to capture interacting proteins both to test our hypothesis that FBXL17 is the cognate E3 ligase but also to identify unknown interactors by mass-spectrometry based proteomics.

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ASBMB

Abstract 1582

Proteostasis Regulators Increases the Surface Trafficking of Pathogenic Mutants in the GluN1 subunit of the NMDA Receptor

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Taylor Benske, Yajuan Wang, Tingwei Mu

N-methyl-D-aspartate receptors (NMDARs) are a class of glutamate-gated ion channels that contribute to the excitatory synaptic response. These receptors are crucial to neuronal communication, synaptic development, and long-term potentiation. The composition of NMDARs is fairly plastic, having two obligatory GluN1 subunits, and two other GluN2(A-D) or GluN3(A-B) subunits. Pathogenic mutations in the GluN1 subunits can result in conditions such as epilepsy, aphasia, intellectual disability, mood disorders, and schizophrenia. One emerging treatment of these disorders involves the use of small pharmacological molecules which can modulate the proteostasis network, a complex system involving the folding, assembly, trafficking, and degradation of proteins. These processes are key for the function of NMDARs, as they must be trafficked to the cell membrane to perform their role, and deficiency in this process is largely associated with the disorders mentioned above. Pathogenic mutations have the potential to disturb this process due to conformational changes, leading to a loss in surface expression, which can result in large insoluble protein aggregates. We aim to restore the surface expression of these pathogenic mutants using proteostasis regulators to upregulate the forward trafficking of GluN1 subunits. In this study, we will explore the ability of select proteostasis regulators to restore the protein trafficking of various loss-of-function pathogenic mutations seen in the GluN1 subunit associated with diminished surface trafficking. Six disease associated variants(DAVs) will be generated, all varying in spatial location and severity. Several proteostasis regulators will be screened, with the most promising compounds in terms of efficacy and application across multiple DAVs being selected for further mechanistic study. We have found that one of the compounds, PR4, is especially potent in both increasing the expression and downregulating the formation of insoluble aggregates of the GluN1 (C744Y) mutant. Multiple assays will then be performed to evaluate the effects of these compounds on NMDAR surface expression and functionality, including but not limited to surface biotinylation assay, immunofluorescence confocal imaging and automated whole cell patch clamping. Through these processes, new therapeutic strategies for neurological conditions resulting from pathogenic mutations of NMDARS could emerge, rescuing these variants through treatment of the proteostasis regulators identified.

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Abstract 1584

Chemoselective caging of carboxyl groups for on-demand protein activation with small molecules

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Clair Gutierrez, Ronald Raines

Tools for on-demand protein activation can enable impactful gain-of-function strategies in biological systems. Thus far, however, proteins have been "caged" at only four amino acids: Lys, Cys, Tyr, and Ser, typically through the incorporation of unnatural amino acids. Here, we report that the preferential reactivity of diazo compounds with protonated acids can be used to expand this toolbox to solvent-accessible carboxyl groups. As a model protein, we employed lysozyme (Lyz), which is a well-characterized enzyme that has an active-site glutamic acid residue (Glu35) with a pKa of 6.2. Mass spectrometry and kinetic assays confirmed that a diazo compound with a bioorthogonal self-immolative handle esterified Glu35 selectively, inactivating Lyz. The hydrolytic activity of the caged Lyz on bacterial cell walls was efficiently restored with two distinct small-molecule triggers. The decaging was more efficient by small molecules than by esterases. Our approach provides unprecedented opportunities for manipulating the activity of native proteins through the reversible caging of their carboxyl groups.

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Abstract 1587

Using Limulus Amebocyte Lysate (LAL) to Discover the Presence of Endotoxins in the Tanks at the Pritzker Marine Biology Research Center

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Endotoxins are harmful for many organisms, including those that live in the water. Therefore, it is important to identify effective ways in identifying if they are present in water environments. Research has been conducted to show that Limulus amebocyte lysate (LAL) is an effective way to determine if endotoxins are present. In this study, LAL was used as the main reagent to detect if endotoxins were present in the tanks at the Pritzker Marine Biology Research Center. It is believed that LAL will be able to detect the presence of any endotoxins within the tanks at Pritzker. The first part of the procedure to do this was to collect 5 mL of water from each tank at Pritzker. After the LAL reagent was dissolved in LAL Reagent Water, the gel-clot assay was then produced. 0.1 mL of the reagent was added to each assay tube. 0.1 mL of each product sample or control was transferred into each assay tube, beginning with the negative control and ending with the highest endotoxin concentration. The contents of the tubes were mixed and incubated undisturbed in a 37 C heating block for 60 minutes. After incubation, each tube was examined for gelation. A positive test is defined as the formation of a firm gel capable of maintaining its integrity when the assay tube is inverted 180 degrees. A negative test is characterized by the absence of gel or by the formation of a viscous mass which does not hold when the assay tube is inverted. Endotoxins were detected in an average of 13 tanks at the Pritzker. To expand upon these findings, quantifying how many endotoxins are present in the tanks and what types of endotoxins are present in the tanks will be tested.

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Abstract 1588

The Measurement of Nitrates and Phosphates in the Sarasota Bay

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The purpose of this study was to measure the nitrate and phosphate levels in the Sarasota Bay and determine if there was a relationship between the nitrate and phosphate levels and location. First, 30 mL of water was collected from the surface and 30 mL of water was collected from the bottom, using the horizontal water bottle sampler, for each of the 28 test sites, for a total of 56 centrifuge tubes. 0.105 mL of sodium hydroxide was added to each tube to preserve the samples. The samples were then analyzed for phosphate and nitrate levels using the Hanna Checker for Nitrate and the Hanna Checker for Phosphate. To analyze the phosphate levels, 10mL of the seawater sample was added to a cuvette and placed into the checker for calibration. After the powder from the reagent packet (HI713-0) had dissolved in the samples, the cuvette was placed into the checker and left for 3 minutes. To analyze the nitrate levels, the same procedure was performed with the nitrate reagent packet (HI782-0) and it was left for 7 minutes in the nitrate checker. Preliminary results show that the average amount of nitrate was 0.0 ppm and that the average amount of phosphate was 0.0096 ppm. The results may be reading 0.0 ppm for nitrate due to the detection limit of the checker not being low enough. However, this data is still useful because as data continues to be collected, if the nitrate levels get high enough to be detected, the reason for the significant difference can be further researched. Further research is being conducted to check how nitrate and phosphate levels change during the day, week, and month at one location. The relationship between macroalgae populations and nitrate and phosphate levels will also be researched.

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ASBMB

Abstract 1595

Formononetin isolated from Sophorae Flavescentis inhibits B cell-IgE production by regulating ER-stress transcription factor XBP-1

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Nan Yang, Xiumin Li

Rationale: Immunoglobulin E (IgE) plays an important pathologic role in allergic conditions including asthma. Studies have shown that ASHMI (anti-asthma herbal medicine intervention) can be used to suppress the production of IgE *in vivo* and in clinically in human. However, the active compounds in ASHMI responsible for the IgE suppression is still unknown.

Objective: We sought to identify the compound (s) in ASHMI that are responsible for IgE inhibition as well as investigate the mechanisms by which the identified compound (s) decreases IgE production.

Methods: The compounds in Sophorae Flavescentis were separated using Column chromatography and preparative-HPLC. The separated compounds were identified using LC-MS and 1H - NMR. U266 cells, an IgE-producing plasma cell line, were cultured with various concentrations of identified compounds. The concentration of IgE produced by the U266 cell culture was measured by ELISA. Trypan blue exclusion was used to determine the number of viable cells from the cell culture. The gene expression of XBP1 and IgE-heavy chain was determined by RT-PCR.

Results: A single compound identified as formononetin was isolated from Sophorae Flavescentis. Formononetin decreased the IgE production in U266 cells across a concentration range of 2 μg/mL to 20 μg/mL without cytotoxicity with an IC50 value of 3.43 μg/mL. Formononetin decreased the gene expression of XBP1, and IgE-heavy chain ($p < 0.001$).

Conclusions: Formononetin decreased IgE production in human B cell line U266 cells in a dose dependent fashion through the regulation of XBP1 ER transcription. Formononetin may be a potential therapy for allergic asthma and other IgE mediated diseases.

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Abstract 1596

Translation of nucleoside ribohydrolase enzyme assays for purified enzymes to direct measurement of enzyme activity in *Trichomonas vaginalis* cells using 19F and 13C-edited 1H NMR spectroscopy

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Carlos Ventura, Valerie Deykina, Nickolas Khayan Lontscharitsch, Edina Saljanin, Ari Gil, Madison Canestrari, Maham Mahmood

Trichomoniasis is the most common non-viral sexually transmitted infection, affecting an estimated 275 million people worldwide. The causative agent is the parasitic protozoan *Trichomonas vaginalis*. The emergence of parasite strains resistant to current therapies necessitates the need for novel treatment strategies. Since *T. vaginalis* is an obligate parasite that requires nucleoside salvage pathways, essential nucleoside ribohydrolase enzymes are promising new drug targets. Fragment screening and X-ray crystallography have enabled structure-guided design of inhibitors for two of these enyzmes, uridine nucleoside ribohydrolase and adenosine/guanosine preferring nucleoside ribohydrolase. Linkage of enzymatic and antitrichomonal activity would be a transformative step toward designing novel, mechanism-based therapeutic agents. While a correlation with inhibition of purified enzyme would be mechanistically suggestive, a correlation with inhibition of in-cell enzyme activity would be definitive. To demonstrate this linkage, we translated our NMR-based activity assays for purified enzymes to direct measurement of enzyme activity in *T. vaginalis* cells. The 19F NMR-based activity assay for the pyrimidine-specific enzyme translated directly to in-cell assays. The spectral editing afforded by 19F observation results in the detection of only the desired nucleoside substrate and nucleobase product signals. However, the 1H NMR-based activity assay for the purine-specific enzyme required a switch from adenosine to guanosine substrate and the use of 13C-editing to resolve the substrate 1H signals from cell and growth media background signals. The in-cell NMR assays are robust, and have been demonstrated to provide inhibition data on test compounds. Potent inhibition of in-cell enzymes will simultaneously establish the mechanism of action and demonstrate cell permeability.

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Abstract 1598

Monitoring the Local Conformations of G-Quadruplexe Structures Using CD-Active Fluorescent Base Analogues

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Stable G-quadruplex (GQ) structures can inhibit increased telomerase activity which is common in most cancers. Usually, spectroscopic methods and thermal denaturation properties are used to evaluate the global structure and the thermal stability of the GQs. The conformational changes to GQ structures during protein and small molecule interaction are more prominent at the local rather than the global structural level. This is because, in spectroscopic methods, signals from the global structures hinder those from the local structure. In this study, we monitored the local conformations of individual G4 layers in GQs using 6-methylisoxanthopterine (6MI) chromophores, which are circular dichroism (CD)-active fluorescent base analogues of guanine, as local conformational probes. A synthetic, tetramolecular, parallel GQ with site-specifically positioned 6MI monomers or dimers was used as the experimental construct. Analytical ultracentrifugation studies and gel electrophoretic studies showed that properly positioned 6MI monomers and dimers could form stable GQs with CD-active fluorescent G4 layers. The local conformation of individual fluorescent G4 layers in the GQ structure was then tracked by monitoring the absorbance, fluorescence intensity, thermal melting, fluorescence quenching, and CD changes of the incorporated probes. Overall, these studies showed that site-specifically incorporated fluorescent base analogues could be used as probes to monitor the local conformational changes of individual G4 layers of a GQ structure. In the future, experiments will be performed on natural GQ-forming sequences to explore the details of small molecule–GQ interaction at the level of the individual G4 layers.

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Abstract 1611

NanoLuc Reporter Cells for Analyzing Antibody dependent cellular phagocytosis of Cancer Cells by Macrophages

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Antibody dependent cellular phagocytosis (ADCP) by macrophages eliminates pathogens and cancer cells. New strategies, including immune modulation, are needed to enhance ADCP and improve the efficacy of anti-cancer therapeutic antibodies and new tools are needed for the measurement of ADCP. ADCP can be measured using luciferase reporters expressed in B lymphoma cells where the luminescence emitted is proportional to the number of living cells. The first luciferase reporter used for quantifying ADCP was firefly luciferase. Firefly luciferase converts the substrate luciferin to oxyluciferin and light in the presence of ATP and oxygen. As firefly luciferase depended on metabolic state of the cell (ATP), this could affect the proportionality of the luciferase signal with the number of viable cells. Moreover, firefly luciferase is relatively dim, required upwards of 104 target cells per well to achieve quantification on a luminescence plate reader. Here we have tested NanoLuc as a reporter for ADCP. NanoLuc, as it does not depend on the ATP or the metabolic state, was then tested and compared to firefly luciferase. NanoLuc was brighter than firefly luciferase, requiring only 102 or fewer target cells per well. However even though NanoLuc produced a bright luminescence, it displayed an exponential decay with a time constant of minutes. We devised a robust mathematical correction to normalize all wells, allowing a full 96 well plate to be read after a bolus addition of the substrate. Moreover, we tested a slowly penetrating substrate, Vivazine, and found that this substrate enabled data acquisition over long periods of time, eliminating the need for the mathematical correction. NanoLuc luciferase produces a large amount of luminescence making it more sensitive than firefly for measuring ADCP. With the corrections, the NanoLuc luciferase reporter is very promising to obtain sensitive and accurate quantifications of antibody dependent cellular phagocytosis.

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Abstract 1623

Ameliorative Effect of *Justicia Secunda* on Liver Function Studies on N-Acetyl-P-Benzoquinone Imine Induced Hepatotoxic Rats

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Ameliorative Effect of *Justicia Secunda* on Liver Function Studies on N-Acetyl-P-Benzoquinone Imine Induced Hepatotoxic Rats Ifeoma J Okpara1, Chidi I Nosiri2, Kizito C Okonkwo3, Ebere L Mbanaso6, Ogechi E Amamasi5, Atasie C Okechukwu 2,Mary O Iwuagwu 7,Constance Nwadike4 1Health, Safety and Environment Department, NSITF, Abuja, Nigeria 2Department of Biochemistry, Faculty of Biological Sciences, Abia State University, Uturu, Nigeria 3Department of Bioscience and Aquaculture, Nord University, Bodo, Norway 4Department of Medical Biochemistry, Imo State University, Owerri, Nigeria 5 Department of Biochemistry, Faculty of Science, Imo State.Owerri. Nigeria 6 Department of physiology, Faculty of Basic Medicine and Health Sciences, Abia State University, Abia State. Nigeria 7 Department of Plant sciences and Biotechnology, Faculty of Biological Sciences, Abia State University,Uturu, Abia State. Nigeria ABSTRACT The liver function Profile of hot water extract of *Justicia secunda* leaf on N-acetyl-p-benzoquinone imine (NAPQI) induced hepatotoxic rats was investigated. Overdose of NAPQI, a reactive metabolite of Paracetamol (PCM) has been reported to cause oxidative stress leading to hepatic injury. The use of plant extracts for the treatment of toxic hepatic diseases, have been largely anchored in traditional medicines. *Justicia secunda*, a plant traditionally used as a tonic due to its blood boosting potential, anti inflammatory, antinociceptive and antioxidant activities was evaluated for its hepatoprotective activities.. This investigation was carried out using 4 groups of 10 rats each comprising of GP1: Control given n-saline, GPs 2–4: given a single oral dose of 2 g/kg body weight each then GPs 3 and 4: administered with 200 and 400 mg/kg HWEJS daily for 7 days. Food and water were given ad libitum. On the last day, the animals were fasted overnight and then anaesthetized with chloroform inhalation and sacrificed. Whole blood was obtained by cardiac puncture and analyzed for liver function profiles. Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP)and Total Bilirubin (TB) levels of the PCM only treated group increased but with significant levels ($P < 0.05$) on ALT and AST relative to the control and the extract treated too. The HWEJS treated groups reduced the levels of AST, ALT, ALP and TB significantly ($P < 0.05$). The Plasma Proteins: Albumin (ALB), Globulin (GLO) and Total Protein (TP) showed slight increase in levels dose dependently compared to the control with the lowest increase on the PCM only treated group. Hepatotoxicity is not due to the drug (PCM) itself but the toxic metabolite (N-acetyl-p-benzoquinone imine (NAPQI)) produced by cytochrome P-450 enzymes in the liver. In normal circumstances, this metabolite is detoxified by conjugating with glutathione in phase 2 reaction. In an overdose, a large amount of NAPQI is usually generated, which overwhelms the detoxification process of reduced Glutathione (GSH) leading to liver cell damage. As indicators of liver injury, AST and ALT elevated levels (on the PCM treated groups only) were reduced by the treatment with HWEJS. These reductions may suggest the hepatoprotective activity of HWEJS especially with the ALT which is a liver specific enzyme. This extract may also have prevented the release of hepatic enzymes. Among other medicinal activities of *Justicia secunda*, this investigation has shown that it exhibits ameliorative effect on liver damage induced by N-acetyl-p-benzoquinone imine (NAPQI) in albino rats.

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Abstract 1625

Towards Tuberculosis control: A Trehalose-based Detection System for live *Mycobacterium tuberculosis*

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Tuberculosis (TB) is second only to COVID-19 as the most lethal cause of death from a single infectious agent. Current primary methods for diagnosing TB infection present significant limitations such as lengthy time-to-result for phenotypic tests, the need for a priori knowledge of *Mycobacterium tuberculosis* (Mtb) resistance mutations, and prohibitive cost for molecular tests. Here, we present fluorogenic solvatochomic trehalose probes that enables rapid detection of live Mtb. In particular, we designed a 4-N,N-dimethylamino-1,8-naphthalimide–conjugated trehalose (DMN-Tre) probe that undergoes >700-fold increase in fluorescence intensity when transitioned from aqueous to hydrophobic environments. This enhancement occurs upon metabolic conversion of DMN-Tre to trehalose monomycolate and incorporation into the mycomembrane of Actinobacteria. DMN-Tre labeling enabled the rapid, no-wash visualization of mycobacterial and corynebacterial species without nonspecific labeling of Gram-positive or Gram-negative bacteria. DMN-Tre labeling was detected within minutes and was inhibited by heat killing of mycobacteria. Furthermore, DMN-Tre labeling was reduced by treatment with TB drugs, unlike the clinically used auramine stain. Lastly, DMN-Tre labeled Mtb in TB-positive human sputum samples comparably to auramine staining, suggesting that this operationally simple method may be deployable for TB diagnosis.

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Abstract 1633

The biogenic amine 3-hydroxy-L-kynurenamine is a metabolite from an alternative branch of Trp pathway and has immunomodulatory activities

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Tryptophan is an essential amino acid, and its derived metabolites play key roles in diverse physiological processes. Three major Trp metabolic pathways have been described: synthesis of serotonin; synthesis of melatonin; generation of kynurenines. In mammalian cells, 90% of Trp is processed through the kynurenine pathway. Kynurenine metabolites are regarded as one of the most powerful mechanisms for immune regulation. They serve as important signaling functions in inter-organ communication and modulate endogenous inflammation. Depending on the cell types, these metabolites can elicit both hyper- and anti-inflammatory effects. Herein we report the major research avenues leading to the characterization of a novel biogenic amine, 3-hydroxy-L-kynurenamine (3HKA), generated through a still uncharacterized lateral pathway of Trp catabolism. This novel biogenic amine is generated by both professional (Dendritic Cells) and non-professional (Lymphatic Endothelial Cells) antigen presenting cells. Differently from all others, Trp metabolites display unique "*in vivo*" immunosuppressive capabilities. *In vitro*, 3-HKA exhibited an anti-inflammatory profile by inhibiting the IFN-g-mediated STAT1/NF-kB pathway in both mouse and human dendritic cells (DCs) with a consequent decrease in the release of pro-inflammatory chemokines and cytokines; most notably, TNFa, IL-6, and IL12p70. *In vivo*, 3-HKA exerted protective effects in an experimental mouse model of Psoriasis by decreasing skin thickness, erythema, reducing TNFa, IL-1b, IFN-g, and IL-17 production, and inhibiting generation of effector CD8+ T cells. Similarly, in a mouse model of nephrotoxic nephritis, besides reducing inflammatory cytokines 3-HKA improved proteinuria and serum urea nitrogen, ameliorating immune-mediated glomerulonephritis, and renal dysfunction. Label free quantitative phophoproteomics of human-monocytes (CD14+) derived dendritic cells and bioinformatics analysis identified up- and down-regulated phosphosites on key signaling transduction receptors, e.g., IFNR1, and other signaling molecules from the JAK/STAT and PKA pathways, including, PKA, CREB, JAK-1 and STAT-1 that could be involved in the regulation of 3HKA anti-inflammatory activities in dendritic cells. In summary, we propose that this novel biological amine is a crucial component of Trp-mediated immune tolerance.

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ASBMB

Abstract 1664

Impact of composition of different Tannic acids towards tannic acid cross linked Zein protein nanoparticles as a delivery system for Curcumin and Metformin

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Tannic acid is a natural plant polyphenol with many biological activities ranging from protein binding to antioxidant activity. Tannic acid is a hydrolysable tannin, comprising a central polyol molecule esterified with one or more galloyl residues. The nominal molecular formula of commercial tannic acid (C76H52O46) suggests the material is decagalloyl glucose, obscuring its complex composition as a mixture of galloyl esters of glucose or other polyols. Owing to its many abundant hydroxyl groups tannic acid forms complexes with proteins based on interactions such as hydrogen bonding and hydrophobic forces. Proteins that are highly amphiphilic and have low water solubility can self-assemble into nanoparticles. Plant proteins are known to be particularly attractive as potential delivery agents for pharmaceuticals due to their minimum potential to provoke zoonotic disease transmission. However, protein nanoparticles exhibit drawbacks such as low stability under gastrointestinal conditions, so strategies including surface coating or cross linking have been employed to improve delivery potential. In the current study, tannic acid is used as the crosslinking agent to synthesize zein nanoparticles that carry curcumin, a hydrophobic polyphenolic supplement extracted from the herb *Curcuma longa* (turmeric) or Metformin, a diabetes medication. Although tannic acid has previously been employed to stabilize protein nanoparticles, the role of the chemical and structural variability characteristic of various preparations of tannic acid has not been considered. In this study we characterize different supplies of tannic acid using reverse phase HPLC and synthesize and characterize zein nanoparticles loaded with curcumin or metformin but coated with different tannic acids. We analyze our nanoparticles with DLS, Zeta potential, SEM and TEM. *In vitro* digestion by chymotrypsin is analyzed by electrophoresis. By successful completion of this project, we hope to contribute to the knowledge pool required to synthesize more stable, even and reliable protein nanoparticles for therapeutic and medicinal purposes.

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Abstract 1668

Mechanism of Neonatal Insulin Production Failure in Transcription Factor PDX1 Related MODY4

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Maturity-onset diabetes of the young (MODY) is a rare autoimmune disorder. Specifically, MODY4 is a subset of cases stemming from mutations in the proline-rich transactivation domain of the homeobox transcription factor PDX1. This study analyzes the mutation PDX1P33 T which substitutes a threonine in the place of a proline in the transactivation domain (Wang, et al). Within pancreatic β-cells, PDX1 is essential for the transcription of the gene INS, which encodes insulin. Further, because of their respectively necessary or augmentary roles in RNA Polymerase (Pol II) recruitment, PDX1 requires the physical binding of cofactors p300 and PSMD9 in order to achieve adequate transcription levels (Qiu et al., 2002, Usher and Showalter, 2022). The PDX1P33 T mutation has been characterized by an inadequate transcription of the INS gene, although the physical process has not been documented. From observations of protein modeling and folding tools PyMol and Alphafold2, respectively, the Mahtomedi MaPS Team, in collaboration with the Center for BioMolecular Modeling, suggests that PDX1P33 T fails to bind PSMD9 and p300 and thus cannot recruit Pol II itself and transcribe INS because of the removal of proline's atypical sidechain via the substitution of threonine; the substitution relaxes the turn radius of a loop in the transactivation domain from 34.78 ± 5° to 79.68 ± 5° (PyMOL, Jumper et al., 2021). As a result, PDX1P33 T leads to MODY diabetes by tangentially inhibiting β-cell development and their capacity to form insulin, as well as the production of insulin itself.

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Abstract 1671

HDI is the Key to a (PICS)ture Perfect Life: The Role of High-Dose Insulin in Combating Poison-Induced Cardiogenic Shock

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Every year over 30 000 people are affected by Poison-Induced Cardiogenic Shock (PICS) as a result of Beta-Blockers and Calcium Channel Blockers. PICS can be treated by High-Dose Insulin(HDI) administered through a central venous catheter. After entering the heart, insulin is able to send signals to Insulin Receptor Substrate(IRS) 1 and 2 through insulin receptors, consisting of subunits a and b linked by disulfide bonds. Then, IRS 1 and 2 are phosphorylated by activated receptors on multiple tyrosine residues that form binding sites for intracellular molecules containing Src-homology 2(SH2) domains. Thus, giving IRS 1 and 2 the ability to bind with the SH2 subunit of the phosphoinositide 3-kinase(PI3 K) pathway. IRS 1 and 2 bind to a pocket of mainly hydrophilic amino acids in the SH2 subunit of the PI3 K pathway. The primary interactions include bonds between phosphotyrosine and serine. Additionally, there are some bonds between hydrophobic amino acids in the pocket including an interaction between proline and glutamine. These bonds between IRS 1 and 2 and SH2 are important because they precipitate further interactions that take place, to ultimately save the heart and the patient from PICS. From there, a resulting increase in PI3 K products promotes translocation of Glucose Transporter Type 4 (GLUT4) to the cardiomyocyte cell membrane, stimulating cardiac glucose uptake. This process is what allows for the heart's exclusive use of glucose as an energy source, which makes the heart return to homeostasis. The Mahtomedi Center for BioMolecular Modeling MAPS Team used 3-D modeling and printing technology to investigate binding sites of IRS 1 and 2 in complex with the SH2 subunit of the P13 K pathway and insulin receptors, in order to understand how HDI can save a patient suffering from PICS.

We would like to thank the Mahtomedi Area Education Foundation for their continued support of our program.

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Abstract 1672

High-Quality Plasmid DNA Purification in 9 minutes following innovative FastFilter Technology

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Traditional methods for isolating plasmid DNA often involve several centrifugation steps, making the isolation process cumbersome and time consuming. Omega Bio-tek's E.Z.N.A.® FastFilter Plasmid DNA Mini Kit is designed to purify high-quality plasmid DNA from up to 5 mL of culture in just 9 minutes. This Kit utilizes the first-of-its-kind FastFilter mini column nested within a HiBind® silica spin column to combine lysate clearance and DNA binding into one simple centrifugation step, skipping the inconvenient cellular debris pelleting and transfer step. The protocol processing time is further reduced by eliminating multiple centrifugation steps without compromising the quality of the purified plasmid. Here, we compare the performance of the Omega Bio-tek's Kit to comparable kits from other manufacturer's and show sequence-quality plasmid purification with similar or better processing times based on number of preparations following Omega Bio-tek's Kit. We elucidate that our kit is capable of handling diverse samples inputs across plasmid types, culture medium, and input culture volume. We also illustrate the downstream suitability of the purified plasmid through sequencing data and restriction digestion analysis. Omega Bio-tek's E.Z.N.A.® FastFilter Plasmid DNA Mini Kit provides rapid and straightforward solution for plasmid purification that is suitable for a wide variety of downstream applications such as sequencing, restriction enzyme digestion, transformation, PCR, and routine screening.

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Abstract 1680

Is Understanding Alzheimer's as Easy as (Aβ) C?: The Role of Aβ and APOE4 in Alzheimer's Disease

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Alzheimer's Disease (AD) is a chronic brain disorder that slowly destroys a person's memory and thinking skills. Researchers have found a correlation between brain insulin resistance (BIR) and AD, along with there being a major genetic risk for AD if a person has the apolipoprotein type 4 (APOE4) genotype. Insulin resistance is caused by impaired function of the GluT4 transporter. Insulin stimulates the exocytosis of GluT4, which enhances its redistribution, and consequently an increased glucose uptake. As the GluT4 attempts to reach the surface of the cell membrane, Amyloid Beta proteins (Aβ) cluster in the neuron synapses after they bind with APOE4. GluT4 needs to transport signals through the Phosphoinositide 3-kinase (PI3 K) pathway, but is unable to reach the synaptic cleft because the Aβ clusters block its path. In a brain unaffected by BIR, the glucose transported by the GluT4 allows neurons to transduce signals involved in memory processing and regulation. Because the binding of APOE4 inhibits GluT4 and causes BIR, as well as the signs of impaired memory seen in Alzheimer's Disease, we continued to explore this established connection in order to distinguish their connection at an intermolecular level. Our research and 3D modeling using Jmol suggest a binding site in APOE4 at hydrophobic amino acid binding region (I) 137–138. We suggest that this corresponds with Aβ's hydrophobic amino acids 17–21, giving them a great affinity to bind with any of the corresponding hydrophobic amino acids in the APOE4 binding site I. This same logic applies to the APOE4 positively charged binding region (II) 145–147, likely to bond to the Aβ negatively charged region 22–23. We can infer that these binding sites may have the strongest possibility of binding due to the compatible chemical properties of each site. Based on our findings, we propose further investigation into how Aβ binds with APOE4 in the brains of Alzheimer's patients in order to target these mechanisms, and their concurring detriments on the brain's function. The Mahtomedi Center for BioMolecular Modeling MAPS Team used 3-D modeling and printing technology to examine structure-function relationships of APOE4 and Aβ in AD patients. This visual model will be a valuable tool in developing our story.

We would like to thank the Mahtomedi Area Education Foundation for their continued support of our program.

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Abstract 1683

Brain Tumor Radio-Sensitization by Novel Mn (III) Porphyrin-based Superoxide Dismutase Mimics

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Cationic manganese(III) porphyrins (MnPs) are among the most powerful superoxide dismutase (SOD) mimics. These compounds were shown to react with numerous other reactive species (such as ONOO-, H2O2 and ascorbate). Cationic MnPs are also able to oxidatively modify activities of numerous proteins, major transcription factors and kinases, engaging them in their mechanism of action both in normal and in cancer cells. The lead Mn porphyrin, MnTnBuOE-2-PyP5+ (BMX-001) is currently in four Phase II clinical trials, including on patients with glioma and multiple brain metastases, as a radio-protector of normal tissues and radio-sensitizer of tumor. Although highly efficacious, the dosing and therapeutic window of BMX-001 are limited due to several side effects they possess: arterial hypotension and mutagenic potential. The molecular basis for MnP-induced side effects is not fully understood, but likely associated with the long peripheral chains and high DNA binding affinity due to penta-cationic charge. In this study, we have explored the radio-sensitizing properties of a new class of asymmetric MnPs on mouse brain tumor model. These structurally modified compounds have been shown to have enhanced brain targeting properties, low systemic toxicity, and improved catalytic redox activity due to reduction of cationic groups and shortening of peripheral chains in porphyrin molecule. The radio-sensitizing properties of novel MnPs were first studied *in vitro* on U87 and GB282 brain tumor cell lines and compared with the clinical candidate BMX-001. The cells were dosed with varying concentrations of MnPs and radiated to study potential synergism on cells using Cell Titer Glo cytotoxicity assay. The synergistic and radio-sensitizing properties of new MnPs were also tested in combination with ascorbate, which was previously shown to potentiate the anticancer effects of MnPs. Among tested MnPs, Mn3 has shown the highest synergism along with enhanced brain-penetration and redox properties. Mn3 has been selected to further investigate its radio-sensitizing properties in a subcutaneous mouse flank tumor model using U87 cells. Mice bearing U87 tumors were randomized to receive (1) vehicle, (2) radiation, (3) Mn3, and (4) Mn3 with radiation. At the end of the study, mouse samples (brain, tumor, liver, muscle, and plasma) were collected to investigate the Mn3 distribution and oxidative stress levels in treated vs non-treatment groups. The study revealed that the combination of Mn3 with radiation significantly reduced the tumor growth as compared to either drug or radiation treatment alone. Studies are in progress to investigate the radio-protective properties of Mn3 on normal

brain in mice. Our initial finding indicate that Mn3 bears significant potential as a new generation of brain-targeting bifunctional agent with tumor radio-sensitizing and normal tissues radio-protecting properties.

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Abstract 1685

The Effects of Environmental PFAS on Honey Bee Health and Human Health Implications of Consuming PFAS-Contaminated Honey Products

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Per- and polyfluoroalkyl substances (PFAS) are a group of anthropogenic aliphatic hydrocarbons in which most-to-all hydrogen atoms have been replaced by fluorine, making them valuable in industry as highly effective surfactants. Their grease- and water-repellent properties also cause PFAS compounds to be extremely environmentally persistent, earning them the nickname of "The Forever Chemicals." The bioaccumulative properties of PFAS compounds have been linked to various health and environmental problems. Honey bees play an important ecological and economical role as pollinators. Additional hive products such as honey and wax also contribute to the global economy, with honey being a preferred sweetener for many. In light of the global pollinator decline, studies on the effects of such a ubiquitous pollutant as PFAS on honey bees and the accumulation of PFAS in honey are crucial. Human consumption of PFAS-contaminated honey products concerns human health, particularly that of children. This review explores the toxic impacts of PFAS compounds on honey bees and other pollinators, the prevalence of PFAS contamination in honey, and the potential PFAS exposure routes for pollinators, including honey bees.

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Abstract 1687

Emerging Treatment for Insulin Resistance using Snake Venom

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The objective of this study is to investigate the enzyme Phospholipase A(2) found in snake venom that could be a treatment for insulin resistant diseases such as Type 2 diabetes, obesity, and cardiovascular disease. Phospholipase A2 works by hydrolyzing the sn-2 (Substitution Nucleophilic Bimolecular) ester bond of a/the phospholipid substrate. The sn-2 reaction is a nucleic substitution reaction where a bond is broken and another is formed synchronously. The PLA2 active site that is responsible for the hydrolysis of sn-2 is located at the bottom of a hydrophobic channel of the C-terminus which has a His-Asp catalytic dyad and a Ca^2+ atom that is responsible for introducing two water molecules into the active site to start the hydrolysis of the phospholipid. The products of the hydrolysis of the sn-2 ester bond of phospholipid are a free fatty acid and lysophospholipid which increases the body's metabolism by generating energy so that the insulin can be used more efficiently throughout the body and prevents insulin buildup. Because of the increased efficiency of the metabolism caused by the PLA2 this process encourages weight loss and prevents weight gain. This emerging treatment of using a phospholipase found in snake venom can create new life changing therapies for those suffering from insulin resistance.

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Abstract 1688

Characterization of severe glaucoma-associated myocilin mutations in an engineered protein scaffold

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Mutations in myocilin are associated with 3–4% of primary open-angle glaucoma, a leading cause of blindness in the world. Mutated myocilin is thought to lead to glaucoma via a toxic gain of function mechanism. Of these disease-associated mutations, ~90% are found in the C-terminal olfactomedin (OLF) domain. Our lab has spent the last 10 years biochemically and structurally characterizing the OLF domain, but the most severe disease mutations have eluded study. Using the principles of protein engineering, we have used a stabilized protein scaffold to enable the study of these severe mutations for the first time. We have used a variety of biophysical techniques including CD, DSF, and protein NMR to determine that even in our stabilized scaffold, these mutations induce changes to the overall structure of the OLF domain. Further structural study will allow us to form new hypotheses about these most destabilized disease mutations lead to such severe aggregation and open the door to new strategies for targeted, personalized treatments.

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Abstract 1708

Cloning and Expression of Amino Acid Ligases for TLN-05220 Biosynthesis

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Katharine Watts

TLN-05220 is secondary metabolite natural product of *Micromonospora echinospora*, a gram-positive bacterium. TLN-05220 exhibits antibiotic activity against resistant pathogens in the same therapeutic range as the last resort antibiotic, vancomycin. It is composed of two distinct structural parts – a multicyclic aromatic scaffold, and a nitrogen-containing piperazinone ring. While the biosynthesis of the hexacyclic aromatic polyketide backbone is generally understood, the synthesis of the unique nitrogen-containing ring remains under investigation. This piperazinone ring consists of glycine, alanine, and serine, which are hypothesized to be ligated to the polyketide scaffold, and one another, by novel amino acid ligase enzymes. We postulate that two asparagine synthetase homologs are involved in ligation reactions. In an asparagine synthetase, a critical N-terminal cysteine catalyzes the release of ammonia from glutamine, which ultimately reacts with aspartate to form asparagine. However, due to a mutation of the N-terminal cysteine to a serine in the homologs, we propose they instead form peptide bonds between two amino acids, and perhaps ligate amino acids to a free carboxylic acid on the polyketide scaffold. In order to further understand the role of these ligases in the biosynthesis of TLN-05220, both amino acid ligases were cloned into the pET28b vector using Gibson Assembly, transformed into *Escherichia coli* BL21, and selected with kanamycin resistance. Both enzymes were successfully overexpressed with an N- and C-terminal 6X histidine affinity tag, and FPLC purified with a Nickel-NTA column. After purification, the ligase yields were found to be 6.89 mg/L and 0.20 mg/L. In the future, the purified enzymes will be tested for ligase activity *in vitro* between various polyketide and amino acid substrates.

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Abstract 1709

Sustained Release of Small Molecules from Nanocomposite Hydrogel Microspheres formed by Aqueous Two-Phase Separation

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Hydrogel microspheres have emerged as a promising solution to the drawbacks of conventional drug delivery due to their tunable physical properties. However, a significant drawback to drug release from hydrogels is a high initial burst release of the drug that disrupts sustained release kinetics and is especially pronounced in the delivery of small molecules. Research has shown that the addition of two dimensional nanosilicate (NS) particles to hydrogel microspheres controls this burst release and improves sustained release of small molecules. The goal of this study was to fabricate NS-containing hydrogel microspheres using an Aqueous Two-Phase Separation (ATPS) method, a type of precipitation polymerization. Hydrogel microspheres were fabricated by mixing 4-arm polyethylene glycol (PEG)-acrylate macromer and PEG-diSH crosslinker solutions with nanosilicate particles and buffer and adding 0.25 M sodium sulfate to form hydrogel microspheres through phase separation. Experiments revealed that the optimal ATPS fabrication conditions were an incubation temperature of 37°C and incubation time of 45 minutes to produce microspheres of ~12 microns in diameter. Microspheres containing a model cationic small molecule, namely acridine orange, were prepared with varying NS concentration to characterize the effect of NS concentration on small molecule release. The results indicate that as NS concentration increases, the initial burst release is minimized, and small molecule release is significantly slower compared to PEG-only microspheres. These findings are significant because the ATPS method allows for facile and high yield fabrication of small microspheres that are around the same size as red blood cells and can easily be injected. Furthermore, the tunable release of small molecules from these microspheres based on NS concentration makes this fabrication method efficient and appealing for drug delivery. Future directions of this study include incorporating chemotherapeutic drugs into NS-containing hydrogel microspheres, and testing the controlled release of chemotherapeutics, injectability of the microspheres, and toxicity against cancer cells.

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ASBMB

Abstract 1712

Synthesis, characterization, and cellular imaging of a novel cyan emitting fluorescent α-amino acid

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The evolution in fluorescent-based approaches for labeling proteins to monitor the biomolecular interactions and protein dynamics is advancing the fields of cellular and molecular biology, biotechnology, and medicine. Among various approaches, small-molecule based approach such as incorporation of fluorescent unnatural α-amino acids (FAA) into proteins for live-cell imaging is attracting wide research interests as they pose no or minimal disturbances in structure and functionality of target protein. Here, we report the synthesis of a novel fluorescent α-amino acid 4-dibenzothiophen-4-yl-L-phenylalanine (DBT-FAA) that emits cyan light in the visible region. The L- α-amino acid DBT-FAA was synthesized using an optimized Suzuki cross coupling reaction in a reasonably good yield. The synthesized DBT-FAA glows cyan fluorescence with a quantum yield 0.74 and is photostable in aqueous solution. Upon incubation with Hela cells, the 4-DBT-FAA at 10 μM gives strong fluorescent signals (upon 405 nm laser excitation) in cytosol with no change in cell morphology. Cells remain normal morphology up to 50 μM DBT-FAA concentration, suggesting no or very minimal toxicity, indicating that synthesized 4-DBT-FAA is biocompatible for live-cells imaging. Further research on site-specific incorporation of DBT-FAA into peptides and proteins to unravel the biological processes for broader applications is underway in the lab. We thank Dr. Y. Wei for technical assistance and the NSF (CHE-1229339), NIH (1R15GM126576-01), and the Orphan Disease Center/Loulou foundation for fundings.

We thank Dr. Y. Wei for technical assistance and the NSF (CHE-1229339), NIH (1R15GM126576-01), and the Orphan Disease Center/Loulou foundation for fundings. The IP issue related to this work is being processed by the OTCV office of University of Massachusetts, MA, USA.

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Abstract 1713

Chemical Space through Transition Metal Catalysis

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Iron-catalyzed reactions, enantioselective reactions, Donor-Acceptor Stenhouse Adducts (DASAs), and strategies for the syntheses of biologically active indole alkaloid natural products are being developed. The significance of these goals cannot be overstated as the discovery of new chemical space leads to improved human health and enables breakthroughs in biology, medicine, and industry. Further, nearly half of approved drugs come from natural products, derivatives, or mimics. Given the early-stage nature of the group, strategies, logic, and preliminary results will be discussed. Specifically, iron catalysts are being used to develop new reactions for the preparation of thioether products. This newly developed method takes advantage of iron's ability to access both single- and two-electron reaction pathways. Secondly, arynes are being used in creative ways to achieve enantioselectivity, a feat that has not been overcome with the highly electrophilic, transient intermediates. Thirdly, new DASA photoswitches have been synthesized and minor structural modifications are resulting in major alterations in DASA physical properties. Lastly, new synthetic strategies to synthesize complex indole natural product, gardflorine A, are being investigated.

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Abstract 1714

Small Molecule Allosteric Modulators of the AML-associated Src-family Kinase, Hck

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Acute myeloid leukemia (AML) is a common form of blood cancer in adults. Overexpression of Src-family tyrosine kinases (SFKs), particularly Hck, Lyn, and Fgr, contributes to AML development and correlates with poor patient outcomes. While ATP competitive inhibitors of Hck and Fgr show promise for AML therapy in cases that overexpress these kinases, their clinical efficacy is potentially limited by the emergence of resistance mutations. Combination therapy for AML using allosteric modulators with ATP-site inhibitors may increase overall inhibitor efficacy while eliminating the potential for acquired resistance mutations compared to monotherapy. This concept was recently shown in a study where combination therapy with an ATP-site and an allosteric inhibitor of Bcr-Abl eliminated the emergence of resistance mutations in a mouse model for chronic myeloid leukemia. To identify allosteric modulators of SFKs, we used a fluorescence polarization assay to detect small molecules that bind to the regulatory region of Hck (unique-SH3-SH2-linker). This effort yielded a series of compounds with a pyrimidine diamine (PDA) core that bind to the Hck regulatory domains as opposed to the ATP-binding site of the kinase domain. In the present study, we mapped the binding site for two of these compounds (PDA1 and PDA2) and determined their effects on Hck kinase activity and overall protein dynamics *in vitro*. In addition, we investigated the effects of these allosteric modulators on the potency of the ATP-site SFK inhibitor A-419259 within a Hck-driven model of AML cell proliferation. Using NMR spectroscopy and SPR, we observed that both PDA1 and PDA2 bind with micromolar affinity to a shared pocket on the surface of the SH3 domain, with possible allosteric effects on the SH3-SH2 connector region. Despite sharing a binding site, *in vitro* kinase activity assays showed that PDA2 enhanced kinase activity while PDA1 had no effect. Hydrogen-deuterium exchange mass spectrometry (HDX-MS) demonstrated that the two modulators have opposing long-range effects on Hck protein dynamics. PDA1 decreased deuterium uptake in parts of the kinase, consistent with stabilization of the closed conformation, while PDA2 increased uptake at kinase domain sites associated with activation. Both compounds reduced deuterium uptake in the SH3 domain, which correlates with the proposed binding site identified by NMR. To test their efficacy in cells, we transformed the human TF-1 myeloid leukemia cell line with a chimeric protein that fused the N-terminal coiled-coil (CC) domain of Bcr to Hck. Introduction of the kinase-active CC-Hck fusion protein rendered TF-1 cells cytokine-independent for growth and sensitized them to the ATP-site inhibitor A-419259, which stabilizes a closed conformation of Hck. PDA1 treatment increased the apparent potency of this ATP-site inhibitor while PDA2 had the opposite effect. Taken together, these data suggest that while PDA1 and PDA2 share a binding site involving the Hck SH3 domain, they have opposite effects as an allosteric stabilizer and disruptor, respectively. Our data support the hypothesis that combinations of allosteric modulators with ATP-site inhibitors may enhance overall potency while reducing the potential for acquired resistance. Our data also suggest that effective combination therapies are dependent on the two compounds having compatible effects on kinase conformational dynamics.

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Abstract 1727

Simplifying the transformation process of *Vibrio natriegens* using the Mix&Go!™ chemically competent kit and Vmax™ X2 purified plasmids

Grace Kelley, *Mercer University*

Jonathan Dudkin, Kerry Strickland

Escherichia coli strains are the most widely used bacteria for molecular cloning and protein expression; however, other options could prove to be more efficient than *E. coli*. A bacteria called *Vibrio natriegens* (V. nat) has the potential to be that promising new tool, as V. nat has a doubling time twice that of *E. coli*. To determine whether V. nat could be more optimal than *E. coli*, this study simplifies the transformation protocol of Vmax™ X2 (a commercially available, genetically modified V. nat) using the Mix&Go!™ chemically competent kit. We then compared the transformation efficiency of BL21(DE3) *E. coli* and Vmax™ X2 with each of the following three plasmids (purified from DH5α *E. coli*): pGST-cloning (expresses his-tagged GST and contains subcloning capabilities), pMSP1D1 (expresses a membrane scaffold protein), and pOGA (expresses O-GlcNAcase). Vmax™ X2 V. nat had the same transformation efficiency as BL21(DE3) *E. coli*. Currently, the transformation efficiency of Mix&Go!™ generated Vmax™ X2 is being improved by transforming with plasmids purified from Vmax™ X2. Simplifying the transformation process could lead to the implementation of a simplified molecular cloning and protein workflow in undergraduate and graduate laboratories.

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Abstract 1730

Optimizing growth of and protein expression in *Vibrio natriegens* (Vmax™ X2) for an improved protein production workflow

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Grace Kelley, Kerry Strickland

Vibrio natriegens (V. nat) has been recently discovered as a promising chassis for molecular biology and biotechnology due to its fast generation time and genetic tractability. Although V. nat has been pushed in the synthetic biology field as a standard chassis for laboratory use in recent years, there are still a multitude of barriers hindering the full potential of V. nat. One of these barriers is the lack of a robust characterized workflow. This study develops a V. nat workflow by determining the optimal media and temperature conditions for protein production in Vmax™ X2, a commercially available genetically modified V. nat chassis. Standard and Mix&Go!™ chemically competent Vmax™ X2 cells were grown in 10 different media (High Efficiency™, autoinduction, autoinduction modified, Terrific, Enhanced 2xYT, 2xYT, LB Luria, SOB, LB Miller and LB Miller with V2 salts) at two temperature (30°C and 37°C) to determine the optimal growth conditions (pre-transformation) and were compared to the growth of BL21(DE3) *E. coli* in 8 different media (LB Miller, autoinduction, autoinduction modified, Terrific, Enhanced 2xYT, 2xYT, LB Luria, and SOB) at 37°C. Protein expression and growth curves were measured for Vmax™ X2 and BL21(DE3) *E. coli* after transformation with each of the following three plasmids: pGST -cloning (expresses his-tagged GST and has subcloning capabilities), pMSP1D1 (expresses a membrane scaffold protein), and pOGA(expresses O-GlcNAcase). Our findings suggest that Vmax™ X2 outperforms BL21(DE3) *E. coli* in growth and protein expression. Future work will include further improvement of V. nat workflow, including plasmid configuration and transformation protocol optimization.

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Abstract 1735

Electrochemical DNA-based biosensors for Bacterial Pathogens: Aptamer discovery, refinement, and adaptation

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Despite advances in antibiotic treatment, bacterial infections are widespread and have serious health consequences, particularly in the developing world. One enduring challenge for bacterial infection is the difficulty in rapidly and easily performing detection and diagnosis outside of hospital and laboratory settings; although PCR and ELISA-based methods are reliable, they require infrastructure not always available in remote, rural settings. Our lab has recently demonstrated a strategy and validated electrochemical DNA-based (E-DNA) biosensor workflow to address this challenge for several bacterial targets relevant to the developing and developed world: Mycobacteria (a source of pulmonary disease), Mycoplasma (lung and genital infections), and Bartonella (Carrion's disease, which causes warts and blindness). Our approach first relies on the generation of DNA aptamers with high affinity towards a cell-surface antigen in the bacterial target. Advances in aptamer selection technology have made this process feasible even for undergraduate level technicians. That aptamer's binding activity is then verified using recombinantly expressed antigen, as well as cultured live bacterial targets. Once verified, the aptamer is adapted into an electrode-bound, internally modified detection system known as E-DNA biosensor. This process involves rational design principles of binding, as well as computational modeling, with the goal of generating a nucleic acid sequence that changes conformation upon binding of the antigen target. The resultant biosensors are field-portable, inexpensive, and provide diagnostic analysis in under 15 minutes. This workflow shows great promise and could readily be expanded to a wider range of targets, providing opportunity to close the gap in detection and diagnosis of bacterial infection in the developing world.

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Abstract 1742

The use of molecular dynamics simulations to study cytokine complexes and its applications for protein engineering

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Type III interferons (IFN-λ1-4) are an important class of cytokines that have antiviral and anti-proliferative properties. They bind to shared receptors– IFN-λR1 and IL-10Rβ – that are co-expressed at barrier tissues, like the lungs and liver, making them attractive, naturally targeted therapeutic candidates. However, their clinical use is still limited due to their lower activity relative to type I interferons, issues with large-scale production, and an incomplete picture of their role in human health and physiology. To better understand this family of proteins, we use a combination of protein engineering and molecular dynamics simulations to study the structural and functional behavior of the IFN-λs and their ligand-receptor complexes. First, we use directed evolution to overcome the low native affinity of the complex by engineering a high-affinity version of IL-10Rβ to enable structural studies for all four wild-type IFN-λ ternary complexes. Secondly, we developed a system to express and isolate IFN-λ4, a recently discovered and poorly understood protein that has been shown to interfere with human immunity. This first-of-its-kind system enables comparative structural and functional studies with IFN-λ1-3. Lastly, in parallel, we use molecular modeling and simulations to describe protein-protein interactions and residue strain fluctuation, produce detailed free-energy landscapes, and reveal previously unknown structural features of the IFN-λ extracellular signaling complexes. We then leverage this information to guide the engineering of the receptor-receptor interface and to identify hot spots of interactions between the ligands and receptors. This two-pronged approach to protein design and engineering reveals valuable molecular insights into IFN-λ behavior and aims to paint a comprehensive portrait of type III interferon structure, function, and signaling that can enable the development of the next-generation of IFN-λ or other cytokine-based therapeutics.

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Abstract 1743

Mycoplasma Detection via Lipoprotein Specific Electrochemical Biosensor

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Aidan Quinn, Audi Fineran, Keaton Silver, Andrew Bonham

Mycoplasma infections primarily effect the respiratory and urogenital systems. Such infections are estimated to impact >2 million people in the United States per year. In addition to human disease, Mycoplasma is a very common source of contamination in laboratory human cell cultures. Current detection methods for such contamination include molecular-based assays, PCR, and serological analysis. These techniques typically take from hours to days to provide results, and require specialized equipment. This creates a critical gap where Mycoplasma infection of cell cultures may go undetected for long enough to jeopardize experimental conditions. In response, we present a method to provide quick, continuous monitoring, and more accurate results using a diagnostic electrochemical DNA-based (E-DNA) biosensor to detect the lipoprotein P48. P48 is a surface antigen shed into surrounding blood serum by most pathogenic Mycoplasma strains into the surrounding blood serum, making it a good target for media-based detection. To enable this strategy, we recombinantly expressed P48 in *E. coli* to serve as a positive control. A modified DNA aptamer that binds to P48 with high affinity was previously integrated into an oligonucleotide scaffold and was used in a gold electrode-bound fashion to give a dose-dependent electrochemical signal change upon binding of the P48 target protein. However, purification of the recombinant P48 protein has proved challenging, as it is poorly soluble and its folded conformation may obstruct N- and C- terminal affinity tagging strategies. Thus, our efforts have been focused on providing a more effective purification method of the recombinant P48, in order to validate our candidate biosensor. If validated to display it's predicted, low nanomolar affinity, and with the known capacity for rapid with continuous sensing, the E-DNA biosensor would serve as a quick and reliable diagnostic tool for Mycoplasma. Such tools can improve prevention, diagnosis, and treatment of Mycoplasma infection in cell culture or in human disease.

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Abstract 1744

Detection of Non-Tuberculosis Mycobacteria (NTM) using Electrochemical DNA Biosensors

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Victoria Colling, Aidan Quinn, Keaton Silver, Andrew Bonham

Nontuberculous Mycobacteria (NTM); is an opportunistic pathogen that is becoming a growing concern for human disease, particularly in tropical and subtropical regions. NTM can cause infections that present similarly to Tuberculosis, known as NTM Pulmonary Disease. The current diagnostic "gold standard" for NTM Pulmonary Disease is a microbial culture-based method, but as NTM are difficult to culture this process can take weeks to months for a definitive diagnosis. To expedite these diagnostic processes of NTM, we detail here a novel diagnostic method using an electrochemical DNA-based (E-DNA) biosensor designed to detect the presence of NTM. To do so, our biosensor targets a surface antigen glycopeptidolipid (GPL), which is NTM-specific and found decorating the cell walls of pathogenic NTM species. The biosensor uses an electrode-bound DNA aptamer specific to GPL, which changes conformation upon binding. This conformational change results in a change to the local environment of a DNA-attached redox tag (methylene blue), and thus can be detected and measured using square-wave voltammetric analysis. One challenge to this process is the need to purify and verify GPL from bacterial lipid extract; this is needed for positive controls and biosensor validation, but isolation and quantification of GPL has required extensive procedure development. Ultimately, this biosensor method will enable the rapid, sensitive, and quantitative detection of GPL. The development of this biosensor has applications in both medical and environmental diagnostics, with our primary goal being the sensitive and rapid detection of NTM pulmonary disease for the purpose of elevated patient care.

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Abstract 1757

Investigation of the synergistic effects of dabrafenib and pterostilbene on melanoma cells

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Melanoma is the most lethal form of skin cancer, and one of the more common cancer types in the United States. Despite the recent advancements in melanoma treatments, very few chemotherapies improve the survivability of patients. To increase the effectiveness of chemotherapies, combinations of different drugs are administered. This combination treatment comes at the added risk of increased toxicity in healthy cells, which causes chemotherapy induced oxidative stress. This study aims to investigate the synergistic potential between dabrafenib, a common treatment for BRAF-mutated melanoma, and pterostilbene, a naturally derived antioxidant. CACL cells, which harbor a BRAF mutation, were cultured in standard conditions and plated in both standard and opaque 96 well plates. The cells were incubated for 24 hours before treatment with dabrafenib, pterostilbene, and a combination of the two. The drug concentrations per well were 10 μM, 30 μM, 60 μM, and 120 μM. After treatment, the cells were incubated for 72 hours. Following incubation, a Presto Blue cell viability assay was performed, and the absorbance and fluorescence data was recorded. In high concentrations (120 μM), pterostilbene alone significantly decreased cell viability by 87.2%. At the same concentration, dabrafenib alone decreased cell viability by 16.2%. In combination, pterostilbene and dabrafenib at 120 μM saw a 67.7% reduction in cell viability. While the combination of pterostilbene and dabrafenib led to a reduction in cell viability compared to dabrafenib alone, the combination of the drugs has yet to demonstrate a synergistic effect, suggesting that optimized concentrations of dabrafenib and pterostilbene will need to be determined. Pterostilbene, while potent, has the potential to be chemically modified to increase its cellular lethality. Future experimentation will implement the use of synthesized novel pterostilbene derivatives with and without dabrafenib. Taken collectively, the results presented here in conjunction with anticipated future results have the potential to precede the development of effective combination therapeutics for malignant melanoma that do not increase oxidative stress.

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Abstract 1766

Group 3 medulloblastoma transcriptional networks are sensitive to EP300/CBP bromodomain inhibition

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EP300 and CBP are paralogous multidomain histone acetyltransferases (HATs) that regulate gene expression. EP300/CBP activity is enhanced in cancer cells, which display increased reliance on the mRNA transcription of tumor-selective oncogenes like the transcription factor c-MYC. This increased reliance in cancer states makes these proteins attractive targets for preclinical therapeutic development. EP300/CBP have multiple domains which can be targeted with small molecules, including bromodomains (BRD) through which they bind to acetylated proteins, and catalytic (HAT) domains through which they acetylate target proteins. The combined activity of the BRD and HAT domains results in high-level transcriptional output, though the relative contribution of these two domains to tumor cell growth is unresolved. To identify specific lineages of cancer cells that display exceptional responses to inhibition of either the BRD or HAT domains of EP300/CBP, we performed a cell viability-based high-throughput screen of 454 different cell lines representing 31 distinct cancer types, using selective inhibitors of the EP300/CBP HAT (A485) and BRD (CCS1477) domains. While most tumor lineages were nearly equivalently suppressed by either BRD or HAT domain inhibition, we identified that the high-risk pediatric brain tumor medulloblastoma (MB) was exceptionally sensitive to BRD inhibition, compared with HAT inhibition. Using biochemical and structural assays, we identified that CCS1477 was highly specific for the EP300/CBP bromodomain, with limited off-target effects on other bromodomain-containing proteins. To examine the acute transcriptional consequences of CCS1477, we performed RNA sequencing for MB cells that were treated with EP300/CBP BRD or HAT inhibitors. As a control, we also treated cells with JQ1, an inhibitor of the related BRD4 bromodomain-containing protein. Gene set enrichment analysis (GSEA) identified that CCS1477 caused selective downregulation of c-MYC-regulated gene sets. Further, western blotting analysis demonstrated that CCS1477, but not A485 or JQ1 treatment, caused an early and selective loss of c-MYC protein. Further, combined network analysis of exome-wide CRISPR-cas9 dropout screening data on 7 MB cell lines, fused with RNAseq data demonstrated that CCS1477 caused downregulation of a dense network of proteins associated with mRNA transcription and splicing, including c-MYC, while A485 and JQ1 did not. These studies identify a selective role for the EP300/CBP bromodomain in maintaining genetic dependency networks in G3MB cells and provide new chemical

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approaches to disrupting malignant transcription in Group 3 medulloblastoma.

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Abstract 1768

Bioactivity and recovery effects of *Xanthium strumarium* L. fruit extract on skin using animal alternative testing

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Jinah Hwang

Xanthium strumarium L. (XS) fruit, as known as a cocklebur, has been used as traditional herbal medicine to treat nasal sinusitis, headache, and arthritis due to its bioactive compounds such as glycosides, phytosterols, and phenolic acids in China, America and Europe. Despite its potential clinical uses, most of the research has focused on its anti-cancer and anti-arthritis effects. Moreover, there are few studies focused on hyaluronic acid production, which consists of extracelluar matix (ECM) molecules and plays an important role in wound healing and skin barrier. The aim of this study is to investigate not only bioactivity such as anti-oxidant and anti-inflammation but also skin protective and recovery properties of XS fruit from Korea and China in human dermal cells, co-culture cells, and skin reconstructed human epidermis (RHE) models. First, anti-oxidant activity of XS fruit was evaluated by DPPH, ABTS and FRAP assay. According to the results, XS fruit grown in Korea (XS-K) showed slightly higher anti-oxidant activity than XS fruit grown in China (XS-C). Based on the lipoxygenase (LOX) inhibition assay, XS-K showed slightly higher LOX inhibition, i.e., anti-inflammatory activity, than XSE-C and 5 mM nordihydroguaiaretic acid (NDGA, a positive control). Moreover, XS-K increased cycloocygenase-2 (COX-2) at the molecular level. Treatment with XS-K and XS-C showed skin recovery potential by increasing mRNA level of hyaluronic acid synthase-2 and hyaluronic acid (HA) production in dose-dependent manners in human dermal cells and co-culture models. In addition, anti-apoptopic effect of XS fruit was confirmed by mitochondrial membrane potential. Both XS-K and XS-C increased mitochondrial membrane potential in a dose-dependent manner in UVB-irradiated HaCaT cells. In contrast, the mitochondrial membrane potential was reduced at the highest dose (50 μg/mL) of XS-K and XS-C, indicating that appropriate amounts of XS fruits may regulate UVB-induced apoptosis. Treatment with XS fruit extract stimulated mRNA level of collagen (COL1A1) but inhibited mRNA level of MMP-1 in human dermal fibroblast, resulting in production and degradation of collagen in ECM. Although both XS fruits exerted skin wound healing effects, XS-K showed more effective wound closure rate than XS-C in skin reconstructed human epidermis model (RHE) as well as human dermal cells and co-cultured model. In conclusion, both XS fruits from Korea and China had anti-oxidant and anti-inflammatory activities, accounting, in part, for protection of mitochondrial membrane potential against UVB-induced apoptosis. Moreover, XS-fruits regulated production and degradation of hyaluronic acid and collagen at the molecular and secretory levels and rapid wound recovery in human dermal cells and RHE models. Collectively, these results

demonstrate that XS fruit may be potentially applied in the area of cosmetic and pharmaceutical industries due to its anti-oxidant and anti-inflammatory activities, wound healing, and skin ECM protective effects.

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Abstract 1771

Inducing an Immune Response in Mice with an mRNA-LNP Dengue Vaccine

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Dengue virus (DENV) causes dengue fever, a disease that is endemic to tropical and sub-tropical regions of the world. Dengue infection ranges from displaying flu-like symptoms to symptoms of severe dengue such as immense bleeding and plasma leakage; severe dengue can lead to hospitalization and death (World Health Organization, 2020). DENV is transmitted between humans and female Aedes mosquitos with an upwards of 390 million dengue infections per year (Bhatt et al., 2013). There are four different serotypes of DENV: DENV1, DENV2, DENV3, DENV4. There is no widely commercialized dengue vaccine, despite the large global burden caused by the virus. Through this study, we investigated the efficacy of the DV2dFL mRNA vaccine, a nucleotide-modified mRNA vaccine, on the immune response of mice when challenged with a live strain of DENV2. This vaccine encodes DENV2 membrane and envelope proteins and is encapsulated in lipid nanoparticles, known as prM/E mRNA-LNP. This study was conducted under guidelines in the Guide for Care and Use of Laboratory Animals of the National Institutes of Health. The Institutional Animal Care and Use Committee (IACUC) at Saint Louis University approved all animal protocols, IACUC (Protocol:2667) and the institutional biosafety protocol IBC (Protocol: 2016-24555). The mouse-adapted DENV2 strain, D2S20, was grown in C6/36 Aedes albopictus cells. Focus forming assays, peptide stimulation, and flow cytometry were utilized to determine the immune response of mice. Successful protection against a lethal DENV2 challenge with no mortality in mice was demonstrated through this study.

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Abstract 1772

Creating conjugate-ready antibody fragments in *E. coli*

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Antibodies and antibody fragments are imperative to the advancement of science and medicine as protein therapeutics, diagnostics, and research tools. Due to their versatility of action and application, optimization of both antibody expression and functionality is of constant interest to researchers and is necessary for the development of new therapeutics. One way to expand the current functionality of antibodies is by site-specifically encoding noncanonical amino acids (ncAAs) with new functionalities through Genetic Code Expansion (GCE). This then eliminates the need to conjugate fluorescent dyes, labels, and drugs to cysteines and unlocks many more sites of incorporation. Here, we wanted to pair the encoding of the rapidly reacting bioorthogonal tetrazine ncAAs with *E. coli* protein expression methods to create homogenous antibody fragments ready for drug- or probe- conjugation. Tetrazine ncAAs serve as an excellent reactive group because they are highly stable, highly selective for their strained-transcyclooctene reaction partner, and most importantly display rapid kinetics enabling complete antibody-conjugation at stoichiometric levels in a manner of minutes. Producing conjugation-ready antibodies in *E. coli* is desirable to lower expression costs, increase the scale of antibody production and hasten production time, which would in turn expand their capacity to be used as medical and scientific tools. This is hindered by the difficulty of overexpressing disulfide bond-containing proteins in the cytoplasm of *E. coli*, due to an overwhelmingly reducing environment. We evaluated the use of solubility tag fusion proteins, various expression conditions and a recently published *E. coli* chaperone and disulfide isomerase system to create soluble antibody fragments, specifically scFV and Fab fragment proteins. We then synthesized this with an improved tetrazine ncAA encoding system to produce tetrazine Fabs and homogenous Fab conjugates. Our research greatly improves the means and accessibility of producing ncAA-containing antibody fragments and enables rapid and complete conjugation of probes to a given antibody site.

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Abstract 1778

The GCE4All Research Center: Unleashing the Potential of Genetic Code Expansion for Biomedical Research

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Kayla Jara, P. Andrew Karplus, Richard Cooley, John Lueck, Bettye Maddux, Ryan Mehl

Genetic Code Expansion (GCE) is a very powerful, but also highly underutilized way to site-specifically place useful chemical groups into proteins. By creating engineered tRNA and aminoacyl-tRNA synthetase (tRNA/RS) pairs specific for non-canonical amino acids (ncAAs) and a repurposed codon, GCE uses the cell's translation machinery to incorporate the ncAAs at genetically-encoded sites during protein synthesis. The GCE4ALL Research Center is a new research center funded by Oregon State University and the Biomedical Technology Development and Dissemination program of the National Institute of General Medical Sciences. The GCE4All Research Center is dedicated to the task of improving GCE tools so that biomedical researchers around the world can easily adopt and use GCE technologies in their own labs to generate specially designed forms of proteins for probing and visualizing how life works. The GCE4All Research Center mission is to improve and disseminate this powerful set of technologies so they can be more widely used for revealing mechanisms of health and disease and developing diagnostics and therapeutics. Research efforts in the Center are organized in two Technology Development Projects focused, respectively, on making optimized GCE tools to incorporate ncAAs relevant for: Bioorthogonal ligations; and Biochemical probes and post-translational modifications. The Center will "road test" developing GCE technologies through formal collaborations with selected researcher groups in the context of authentic, challenging problems in biomedical research – referred to as Driving Biomedical Projects. Our presentation here will provide an overview of the GCE4All Research Center structure, describe our approaches to technology development and dissemination, and introduce the initial set of technologies that we have targeted for improvement and wide adoption. Also, as you have interest, please: Explore our resources at https://gce4all.oregonstate.edu/ Contact us at gce4all-center@oregonstate.edu.

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Abstract 1786

Structural Insights into Inhibition of Human DNA Methyltransferases

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Mammalian DNA methylation is a key mechanism of epigenetic regulation established and maintained by DNA methyltransferases (DNMTs), including DNMT1, DNMT3A and DNMT3B. All DNMTs contain a methyltransferase domain for transferring a methyl group to the cytosine C5 position of CpG sites. To date, various non-covalent DNMT inhibitors have been identified, but the underlying mechanisms for their inhibitory activity and specificity for each DNMT are unknown. Here, several structurally diverse non-covalent DNMT inhibitors were selected for testing their inhibitory activities against recombinant human DNMT1, DNMT3A and DNMT3B. Among these non-covalent DNMT inhibitors, a natural compound, harmine, efficiently blocks the methyltransferase activity of DNMTs. Binding assay using intrinsic tryptophan fluorescence suggests that harmine directly interacts with DNMT3B. Co-crystal structure of the catalytic domain of DNMT3B/DNMT3L in complex with harmine was determined revealing that harmine is bound at the cofactor SAM-binding site. Kinetic assays further confirmed that harmine competes against SAM in inhibiting the activity of DNMT3B. In summary, our study reveals the DNMT inhibition mechanism by harmine and suggests new directions for developing novel DNMT inhibitors as therapeutics for the treatment of diseases related to DNA hypermethylation by DNMTs.

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Abstract 1801

Graphene Quantum Dots as Drug Delivery Platforms for Anti-Cancer Applications

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Prostate cancer (PCa) is the most frequently diagnosed cancer and the third leading cause of cancer mortality in men in the US. (2019) There are many types of cancer treatments such as: Rehabilitation therapy, Immunotherapy, Hormone therapy, Stem Cell Therapy, Chemotherapy, etc. Specifically, chemotherapy has many limitations because most current drugs used also destroy healthy cells and cause: nausea, vomiting, alopecia, diarrhea, anemia, bleeding, sterility, among other side effects. For this reason, we developed nanoparticle (NPs) drug delivery platforms for cancer treatment, composed of silver graphene quantum dots nanocomposites (AgGQD), which are known to enhance drug therapies. Silver nanoparticles (AgNPs) are toxic to both normal and cancer cells. They impart toxicity to cancer cells by decreasing mitochondrial function, production of reactive oxygen species (ROS), release of lactate dehydrogenase (LDH), dysregulation of the cell cycle, induction of apoptotic genes such as Bax, micronucleus formation, chromosome aberration and DNA damage. In addition to these cellular mechanisms, AgNPs have also shown antiangiogenic and antiproliferative properties. AgNPs are antiangiogenic by inhibiting PI3 K phosphorylation of Akt, so the signaling pathway cannot complete, eventually ending angiogenesis, starving the cell of oxygen, and killing the tumor cell. In this study, graphene quantum dots (GQDs) are used to reduce the toxicity of AgNPs in healthy cells and improve their performance as drug delivery platforms. In addition, GQDs may help to increase the solubility of drugs that have shown low solubility in water. To achieve this, the NPs were synthesized using graphene quantum dots as reducing agents. We tested for toxicity in PCa cells (PC-3) and assessed how the AgGQDs affect blood cells, through morphology and hemolysis tests. As a result, changes in blood morphology were observed, while showing low hemolysis.

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Abstract 1810

The efficacy & molecular mechanisms of a terpenoid compound Ganoderic Acid C1 on corticosteroid resistant neutrophilic airway inflammation: *In vivo* & *ex vivo* validation

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Neutrophil predominant airway inflammation is associated with severe and steroid-resistant asthma clusters. Previously we reported efficacy of ASHMI, a three-herb TCM asthma formula in a steroid-resistant neutrophil-dominant murine asthma model and further identified Ganoderic Acid C1 (GAC1) as a key ASHMI active compound *in vitro*. The objective of this study is to investigate GAC1 effect on neutrophil dominant, steroid resistant asthma in a murine model. In this study, Balb/c mice were systematically sensitized with ragweed and alum and intranasally challenged with ragweed. Unsensitized/PBS challenged mice served as normal controls. Post sensitization, mice were given 4 weeks of oral treatment with GAC1 or acute dexamethasone (Dex) treatment at 24 and 48 hours prior to challenge. Pulmonary cytokines were measured by ELISA, lung tissue proteins were assayed by western blot and lung sections were processed for histology by H&E staining. Furthermore, GAC1 effect on MUC5AC expression and on reactive oxygen species (ROS) production in human lung epithelial cell line (NCI-H292) were determined by qRT-PCR and ROS assay kit, respectively. Computational analysis was applied to select potential targets of GAC1 in steroid-resistant neutrophil-dominant asthma. Molecular docking was performed to predict binding modes between GAC1 and Dex with STAT6. Our results showed that GAC1 treatment, significantly reduced pulmonary inflammation ($P < 0.01–0.001$ vs. Sham) and airway neutrophilia ($P < 0.01$ vs. Sham), inhibited TNF-α, IL-4 and IL-5 levels ($P < 0.05–0.001$ vs Sham). Acute Dex treatment reduced eosinophilic inflammation and IL-4, IL-5 levels, but had no effect on neutrophilia and TNF-α production. GAC1 treated H292 cells showed decreased MUC5AC gene expression and production of ROS ($P < 0.001$ vs stimulated/untreated cells) NF-kB activation was inhibited by both GAC1 and Dex, however only lungs from GAC1-treated mice displayed reduced phosphorylation of Akt and STAT6 ($P < 0.05$ for all) and upregulated expression of HDAC2 ($P < 0.05$ vs Sham). Molecular docking results showed binding energy of complex GAC1-STAT6 was −8.4 kcal/mol. GAC1 may be a promising anti-asthma botanical drug for treatment of steroid-resistant asthma.

This study was funded by NIH/NCCAM center grant #1P01 AT002644725-01 “Center for Chinese Herbal Therapy (CHT) for Asthma”, the Sean Parker Foundation “ASHMI Active Compounds for Asthma Therapy”. We also thank Guangzhou ImVin Pharmaceutical Co., Ltd for supporting H Li under a Visiting Scholar program.

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Abstract 1811

Anti-proliferative effects of curcumin, silibinin, and combination of curcumin plus silibinin on gastric cancer

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Epidemiological studies have shown that a diet that is rich in fruits, vegetables, and spices reduces the risk of developing cancer. This effect may be attributable to synergy between phytochemicals (biologically active compounds derived from plants) that are consumed at low levels. Gastric cancer is one of the most common and deadly cancers worldwide and is a leading cause of cancer-related deaths. Risk factors for gastric cancer include tobacco use, alcohol consumption, stomach ulcers, and inflammation caused by *Helicobacter pylori*. Several inherited genetic mutations are associated with an increased incidence of gastric cancer. Regular dietary intake of phytochemicals has the ability to reduce the risk of major cancers. While exposure of cancer cells to a single phytochemical may be less effective in killing them or suppressing their growth, a combination of phytochemicals should lead to additive or synergistic effects. Combination treatments can be effective at low doses, making them candidates to prevent cancer initiation and/or progression. Previously, we have demonstrated a synergistic effect of curcumin and silibinin against colorectal cancer. In the present study, we assessed the anti-cancer effects of curcumin and silibinin individually, and in combination, against the gastric cancer cell line AGS. Our studies indicated that curcumin and silibinin in combination was more effective in inhibiting proliferation in the AGS cell line compared to the individual compounds or vehicle control. Also, curcumin and the combination of curcumin and silibinin exert their anticancer activities via the modulation of the Wnt signaling pathway, and the resulting cell death is mediated by apoptosis.

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Abstract 1818

Development of MGAT1 Inhibitors Based on a Novel High-throughput Screening System to Promote Breast Cancer Immunotherapy

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Introduction: Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer that presents high metastasis rates and poor survivability. Although immune checkpoint blockade (ICB) therapy sheds light on TNBC treatment, only a small fraction of TNBC patients can benefit from it and the drug resistance has become a growing concern. Therefore, there is an urgent need to develop novel targeted therapies synergizing with ICB therapy. Accumulating evidence has demonstrated that N-glycosylation plays a critical role in the anti-tumor immune response; However, very few specific glycosyltransferase inhibitors have been successfully developed due to the structural complexity associated with the glycosylation process and the lack of specific methodologies to study it.

Objective: This project aimed to develop a novel *in vitro* functional-based high-throughput screening system to discover specific inhibitors targeting MGAT1, a glycosyltransferase strongly correlated with cancer progression, metastasis, and immunotherapy unresponsiveness in cancer patients.

Methods: This system incorporates four key components: (1) biosynthesized Man5GlcNAc2Asn glycans specifically recognized by MGAT1; (2) the UDP-N-Acetylglucosamine sugar donor; (3) the UDP-Glo Glycosyltransferase Assay kit which includes UDP detection reagent; (4) functional MGAT1 protein. In *in vitro* conditions, MGAT1 will attach the N-Acetylglucosamine sugar onto the substrate glycan and release UDP into the solution. The UDP concentration will be characterized with UDP detection reagent in the form of luminescence as an indicator of MGAT1 function. However, when an inhibitor interferes with MGAT1 function, less UDP will be freed, and less luminescence will be detected. By detecting the amount of UDP released from the enzymatic reaction, this system can quantify MGAT1 enzymatic efficacy with high sensitivity.

Results: Based on initial dose- and time-dependent experimental data, we have determined the ideal reaction conditions and confirmed that our system is able to convert enzymatic MGAT1 function into luminescence. Utilizing our validated conditions, we conducted a primary screening with 400 compounds and discovered a leading compound (#72) with an IC50 value of 0.64 ± 0.095 uM that may serve as a potential MGAT1 inhibitor.

Conclusions: We have developed a high-throughput screening system that can characterize the catalytic function of MGAT1 with quantifiable luminescence. The preliminary screening has discovered a leading compound that may inhibit MGAT1 enzyme function. The efficacy of leading compounds will be further validated with *in vitro* and *in vivo* studies. On the

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one hand, this system incorporates a novel functional-based approach to discover more promising inhibitors compared to traditional protein-protein or protein-drug interaction systems. On the other hand, this system will be applied to target numerous therapeutic targets found across N-glycosylation pathways.

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Abstract 1820

Progress Towards the Synthesis of a Photoisomerizable Antagonist of the 5-HT2A Receptor

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Craig Streu

5-HT2A Antagonist Serotonin Neuropharmacology, 5-HT2 receptors are G-protein coupled receptors belonging to the serotonin receptor family. There are many different subtypes of 5-HT2, specifically the 5-HT2A receptor, which is found throughout the entire body including the central nervous system and areas in the brain. It is embedded in the plasma membrane of cells and has a significant effect on memory and cognition. Many antipsychotic drugs target this receptor with the goal of treating mood disorders such as schizophrenia and bipolar disorder. Serotonergic hallucinogenic drugs also target this receptor, which results in an altered cognitive state and hallucinogenic effects. Azo compounds are colorful photoswitchable molecules that have a nitrogen-nitrogen double bond between two benzene rings. They can switch their conformation when illuminated by ultraviolet light, which confers a shape change in the corresponding molecule. Such shape changes may result in substantial modulation of drug binding ability, allowing the drug to be reversibly activated and inactivated by light with excellent spatial and temporal selectivity. Sarpogrelate is a known hypertension drug, but it has also been found to bind to the 5-HT2A receptor, acting as an antagonist. This theoretically prevents serotonin binding in the brain and therefore can halt a hallucinogenic experience and be used in treating mood disorders involving too much serotonin binding. Having a photoswitchable version of sarpogrelate should allow for the selective spatial and temporal antagonism of 5-HT2A, allowing exquisite control for the study of neuronal circuitry. We herein disclose the design, computational modeling, and progress toward the synthesis of a photoisomerizable azo compound that targets the 5-HT2A receptor in the brain to create a switchable research probe and antipsychotic drug.

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Abstract 1827

Inhibition of Soluble Epoxide Hydrolase Altered Gut Barrier Function and Gut Microbial Diversity in a Mouse Model of Alcohol-Associated Liver Disease

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Study Objective: Chronic, excessive alcohol consumption leads to numerous adverse health effects in multiple organs including the liver (steatosis, steatohepatitis, and cirrhosis) and gut (impaired barrier function) and can also lead to gut bacterial dysbiosis. Our group and others have recently identified that pharmacological inhibition of soluble epoxide hydrolase (sEH), an enzyme which degrades beneficial signaling lipids, is an efficacious therapeutic strategy for alcohol-associated liver disease (ALD) in mice. While the benefits of sEH inhibition on alcohol-associated pathologies in the liver have been demonstrated, little is known regarding the effects of sEH inhibition on the gut-liver axis, and in particular, on ethanol-induced intestinal permeability and gut bacterial dysbiosis. The objective of the current study was to assess gut barrier function and bacterial alpha and beta diversity in experimental ALD using a mouse model with or without administration of an sEH inhibitor.

Methods: C57BL/6J mice were randomized to receive either an ethanol or control (pair-fed [PF]) Lieber DeCarli liquid diet for 8 weeks. In each diet group, mice received an sEH inhibitor (t-TUCB, 3 mg/kg) in the liquid diet or a vehicle control (n = 7–10 mice per group). Following euthanasia, plasma and fecal pellets were collected from each group of mice (n = 6 fecal pellets per group). Plasma ALT (a marker of liver injury) and soluble CD14 (a marker of endotoxemia) were measured. Fecal genomic DNA was isolated using the Qiagen QIAamp PowerFecal Pro DNA Kit. 1 μg DNA per sample was provided to Azenta Life Sciences for 16S rRNA sequencing. Differences in relative or absolute taxa abundances were calculated by one-way ANOVA using GraphPad Prism 9 software. Principal component analysis (PCA) was conducted in Prism and visualized using R in R Studio via the plot3D function of the rgl package.

Results: Ethanol feeding led to increased liver injury and endotoxemia as measured by plasma ALT and soluble CD14, respectively, which were each significantly reduced by t-TUCB. Analysis of the gut microbiota revealed no changes in bacterial alpha diversity (by multiple endpoints) by either ethanol or t-TUCB. However, PCA revealed a distinct effect of ethanol on beta diversity at all taxonomic ranks measured (phylum through species). t-TUCB administration led to changes in beta diversity as measured by PCA on primarily the phylum and class taxonomic ranks. The changes caused by t-TUCB were more prominent in PF, rather than ethanol-fed, animals. When investigating the specific changes driving differences in beta diversity, we noted that t-TUCB elevated levels of various beneficial Actinobacteria, including order Bifidobacteriales, family Bifidobacteriaceae, and genus Bifidobacterium. t-TUCB also led to increased levels of phylum Verrucomicrobia, class Verrucomicrobiae, order Verrucomicrobiales, and increased genus Akkermansia including *A. muciniphila*. Lastly, t-TUCB elevated *Lactobacillus reuteri*, a beneficial microbe belonging to phylum Firmicutes.

Conclusions: In our experimental model of ALD, sEH inhibition reduced liver injury and endotoxemia and led to numerous changes in the microbiota, including beta diversity at multiple taxonomic ranks, including increases in microbes associated with gut health and homeostasis such as Bifidobacterium, Akkermansia, and Lactobacillus. Future research will elucidate the role of gut bacterial changes in the beneficial role of sEH inhibition in ALD.

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Abstract 1836

Developing Peptide Keys to Unlock Protein Degradation in *C. elegans*

Connor Wakefield, *Wabash College*

Michael Bertram, Adam Berg, Mitchell Keeling, Austin Johnson, Jakob Faber, Walter Novak, Erika Sorensen-Kamakian

Our lab is developing an *in vivo* method to inactivate gene function to better understand development and disease. While gene inactivation using RNAi or genetic mutation allows researchers to reduce or eliminate a gene product, these techniques have limitations, including (1) slow protein turnover, which can impair the use of RNAi, and (2) the inability to characterize essential gene function later in development. As a result, effective methods for conditional protein degradation offer a powerful alternative to gene inactivation. Our lab is developing the de novo 'Latching Orthogonal Cage/Key pRoteins' (LOCKR) technique to control protein degradation in *C. elegans*. LOCKR uses designed protein Switches and Keys. The Switch cages a protein sequence, in this case a degron, in the "locked" state (degronSwitch). Upon addition of the Key, the degronSwitch is "unlocked," exposing the degron and causing degradation of the degronSwitch and any protein fused to it. Our work seeks to optimize and characterize the Key in the *C. elegans* worm. First, we codon-optimized the Key (ceKey) for worms since the original construct was designed and tested in yeast. Second, we designed and generated a template construct of the ceKey by fusing GFP, a 3xMyc tag, and a tubulin 3'UTR into a *C. elegans* MosSCI plasmid using PCR and Gibson cloning. The GFP and 3xMyc tag were added to ceKey for downstream Key visualization via microscopy and Western blotting; whereas, the tubulin 3'UTR was added for mRNA stability. Third, ceKey expression can be tailored using promoters, giving LOCKR the potential for incredible spatial protein control. To this end, the ceKey was placed under the control of tissue-specific promoters. Lastly, we report here the successful creation of ceKey animals via MosSCI and our characterization of ceKey expression. Our future steps include testing Key and Switch interactions *in vivo*.

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Abstract 1838

Analysis of evolutionary variants of a bacterial protein system for the basis of programming dynamic metazoan systems

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It is important for cells to self-organize molecules in space and time, and we are interested in exploring how these functions can be used and manipulated to form complex systems of our own design. In a previous study, using the bacterial proteins MinD and MinE (MinDE) from *E. coli*, we created a programable reaction-diffusion system capable of functioning in mammalian cells. In *E. coli*, the MinDE system functions by forming pole-to-pole oscillations across the cell, which defines the Z-ring position at the midpoint of the cell for division. When expressed in mammalian cells, this system retains its reaction-diffusion behavior and oscillates at a genetically encoded frequency that can be analyzed using fluorescence signals. Additionally, we show the system can be further modified to create circuits or patterning. To better understand how to modulate MinDE for programming, we examined analogous MinDE evolutionary variants from other rod-shaped bacteria that serve the same function with modified spaciotemporal dynamics caused by evolutionary mutations in the protein amino acid sequences. We found it possible to cross-mix MinD and MinE from different species, resulting in altered patterning caused by different affinities between MinD to the cell membrane and MinE to MinD. It is also possible to mix and match components of the proteins, such as their membrane targeting sequence, to create MinDE hybrids with further altered targeting and binding affinities, and consequently behavior. This study lays the groundwork for repurposing other existing protein systems from nature as well as engineering synthetic protein systems with varying dynamics to manipulate endogenous cellular pathways based on our analysis of variants and combinations of the MinDE system.

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Abstract 1839

Constructing De Novo Designed Protein Switches in *C. elegans*

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Austin Johnson, Adam Berg, Joe Kaefer, Connor Wakefield, Jordan Scott, Walter Novak, Erika Sorensen-Kamakian

The ability to inactivate gene function *in vivo* is essential for understanding the molecular mechanisms that regulate normal and disease phenotypes. While genome editing technologies such as CRISPR-Cas9 are effective for generating gene knockouts with constitutive loss of protein product, these technologies cannot be used to generate gene knockout mutants for essential genes, which are required for viability. Essential gene products likely have critical roles later in development and homeostasis, highlighting the need for effective methods to examine these proteins *in vivo*. Recent work to develop methods to conditionally deplete proteins (e.g., AID, ZIF-1, PSD, etc.) have been effective, but these methods are limiting because they use a single input signal to control protein levels, preventing simultaneous differential control of multiple proteins with only one approach. Here we discuss the development of the LOCKR (Latching Orthogonal Cage–Key pRoteins) system for conditional protein depletion in *C. elegans*. LOCKR is a de novo designed protein switch. There are two main components of LOCKR used for protein degradation: (1) the degronSwitch and (2) the inducible Key. Here, the degronSwitch is fused to a protein of interest and, in the absence of Key, is in the off state, caging the cODC degron. Upon the addition of the inducible Key, the Key binds the degronSwitch, "unlocking" the degron. The exposed degron causes degradation of the Switch and the fused protein. Unlike other conditional protein techniques, LOCKR Keys and degronSwitches can be designed as orthogonal pairs, allowing for the differential control of multiple proteins simultaneously. This work focuses on optimization of the degronSwitch for addition at any locus in *C. elegans* via CRISPR-Cas9 gene editing. First, we designed our degronSwitch homology-directed repair (HDR) construct by fusing optimized wrmScarlet and a 3xFLAG tag to the degronSwitch using PCR and Gibson cloning. The wrmScarlet and 3xFLAG tag were added for downstream degronSwitch visualization via microscopy and Western blotting. Second, we created an inert degronSwitch reporter for ubiquitous expression of the degronSwitch *in vivo* to examine the impact of degronSwitch protein expression on worm health. Third, we designed sgRNAs for editing of the 3' end of dynein and synaptotagmin and added the appropriate 120bp overlaps to the HDR constructs. These proteins will test the effectiveness of the LOCKR system on both cytoplasmic and transmembrane proteins. We report here the initial characterization of the degronSwitch *in vivo*, which contributes to the optimization of LOCKR in *C. elegans*.

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Abstract 1857

Developing new strategies for protein engineering by extrapolation of machine learning models

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Engineered proteins are useful in many applications from protein therapeutics to chemical biosynthesis. However, traditional protein engineering techniques are labor, resource, and time intensive. Machine learning is a powerful tool for protein engineering because it can predict the functions of unobserved protein sequences by picking up on patterns between observed sequences and their functions. We have developed an efficient protein engineering pipeline that extrapolates machine learning models trained on protein-function data to discover distant, improved protein variants in silico. In our proof of concept, we found that when trained on only single and double mutants, machine learning models can be extrapolated to find significantly improved protein variants up to ten mutations from wild-type. However, a number of design considerations remain unclear, including which architectures can propose the fittest variants, how far models can be extrapolated to find functional variants, and whether ensembles of models increase design confidence. To examine these questions further, we use the B1 domain of protein G (GB1) for improved binding affinity to Immunoglobulin G (IgG) as a model engineering target. We task a variety of machine learning models to produce GB1 variants with a varying number of mutations that have improved binding affinity for IgG. We synthesize and screen top predicted designs from each condition with yeast display and FACS. We find our engineering strategy is capable of identifying highly fit sequences within a small sequence budget – an important consideration for practical engineering applications.

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Abstract 1860

Modulation of Cellular Proliferation and Antioxidant Defenses by Cerium Oxide Nanoparticle

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Background: Radiation medicine uses high energy photons or particulate radiation to cause cytotoxicity by destroying the DNA and by causing the production of damaging free radicals. There is also unintended bystander effects due to radiation interacting directly with the cellular targets or by free-radical mediated oxidative damage to off-target cells. Cerium oxide (nanoceria) is a well known nanoparticle that acts as a free radical scavenger due to its antioxidant properties. Previous studies of nanoceria showed wide ranging effects from radioprotection in lymphocytes to radiotoxicity in leukemia cells. We investigated effects of nanoceria on cellular health in terms of cell proliferation and antioxidant defenses *in vitro*. We also assessed the effect of nanoceria *in vivo* dermal application on mouse skin.

Purpose: To demonstrate that cerium oxide nanoparticles modulate cell proliferation and antioxidant defenses in breast cancer cells and is safe for dermal topical application in a mice model.

Methods: MDA MB231 cells were cultured in triplicate in 96 well plates at a density of 10 000 cells/well. The cells were treated with 0, 50, 100, or 200 μg/mL cerium oxide nanoparticles, respectively, for 72 hours. The nanoceria treated samples were then either treated with RealTime-Glo™ reagent for cell viability or with GSH-Glo™ Glutathione reagent to assess intracellular glutathione levels. Samples were measured for luciferin fluorescence using a microplate reader at an emission of 560 nm after correcting for autofluorescence. The dorsal skin of mice was depilated in a 1 cm × 1 cm area using a shaving razor and cerium oxide nanoparticles at a concentration exceeding 200 microgram/cm^2 was applied using cotton tipped applicator two times a week for two weeks and observations were made for four weeks.

Results: Our results showed that: (1) There was normal or decreased RealTime-Glo™ fluorescence in cells not treated with nanoceria while increased fluorescence was noted in samples treated with nanoparticles in proportion to the dose. (2) The number of cells exhibiting the oxidation product of glutathione decreased proportionately in samples treated with increasing concentration of cerium oxide nanoparticles, in comparison to the untreated samples. (3) Mouse treated with or without the nanoceria exhibited no visual differences in behavior, skin texture, or appearance up to one month after treatment.

Conclusion: Cerium oxide nanoparticles modulated cellular proliferation and antioxidant defenses in a dose dependent manner in breast carcinoma cells and exhibited apparent safety for dermal applications in mice.

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Abstract 1861

Revealing the nascent proteome by selective labeling, high-sensitivity capture, and mass spectrometry

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Bala Vinaithirthan, Juan Oses-Prieto, Robert Chalkley, Alma Burlingame

Cellular responses to stress, pharmacologic treatments, or environmental cues can trigger rapid alterations in the translatome. Characterization of proteins undergoing translation in response to stimuli is challenging and requires sensitive and specialized methods. Previously, we reported on the use of O-propargyl-puromycin (OPP) to label nascent proteins in a 2-h time frame. After cell lysis, the OPP-tagged polypeptides were biotinylated using click chemistry, captured by affinity purification, subjected to on-bead digestion, and identified by tandem mass spectrometry (OPP-ID). Here, we have incorporated a chemically cleavable linker, Dde biotin-azide, into the protocol to circumvent the problem of high background arising from non-specific binders in on-bead digestions. Following capture on streptavidin-agarose resin, the Dde moiety is cleaved with 2% hydrazine, releasing nascent polypeptides bearing OPP plus a residual C3H8N4 tag. When compared side-by-side with the original OPP-ID method, this change led to a dramatic reduction in the number of background proteins detected in PBS-treated controls with a concomitant increase in the number of proteins that could be characterized as nascent in OPP-treated K562 cells. We also achieved extensive nascent protein detection from protein inputs as low as 100 μg and a modest improvement in protein sequence coverage when using the cleavable linker. As an acute perturbation, we pulsed K562 cells with MLN128, an ATP-competitive inhibitor of the mammalian target of rapamycin (mTOR), for 1 h prior to OPP treatment and compared the nascent proteome to the whole cellular proteome in three biological replicates. Results from these experiments, which revealed strong downregulation of a functional network of nascent proteins upon mTOR inhibition, will be presented.

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Abstract 1863

Characterization of immunoliposomes for HER2-targeted delivery of the dual Rac/Cdc42 inhibitor MBQ-167

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Suranganie Dharmawardhane

Breast cancer is the second leading cause of mortality among women in the US. Among the various subtypes, human epidermal growth factor receptor 2 (HER2) positive breast cancer shows a very aggressive and invasive phenotype. This subtype is characterized by the overexpression of HER2 receptor on the cell surface that upon ligand binding and dimerization, leads to overactive cell signaling that promotes cell proliferation and metastasis. Even though therapies have been developed to treat this type of breast cancer by targeting HER2 and preventing dimerization (eg. Trastuzumab), patients can present with acquired or intrinsic therapy resistance. One of the mechanisms of resistance is the compensation of intracellular signaling from other receptors. This signaling converges on guanine nucleotide exchange factors (GEFs) that activate Rac and Cdc42 by exchanging GDP for GTP, thus activating downstream effectors that modulate the actin cytoskeleton to promote cell migration and invasion. Therefore, targeting Rac and Cdc42 activation selectively in HER2 positive breast cancer is a promising strategy for overcoming HER2-targeted therapy resistance. Previously, we characterized the dual Rac/Cdc42 inhibitor MBQ-167 that inhibits Rac and Cdc42 activity with IC50s of 103 nM and 78 nM, respectively. However, there is a need to develop selective delivery systems that transport MBQ-167 directly into HER2-positive breast cancer cells. Our objective is to deliver MBQ-167 selectively into HER2-positive cells using liposomes coated with Trastuzumab, a clinically used monoclonal antibody that targets HER2. We conjugated Trastuzumab to a lipid linker (DSPE-PEG-Maleimide) by reacting Trastuzumab with 2-iminothiolane (Traut's reagent) under nitrogenated (low oxygen) conditions and then mixing with the lipid linker overnight. This reaction was characterized by measuring the thiol groups formed after the reaction with Traut's reagent and after mixing with the lipid, followed by mixing the Trastuzumab-lipid conjugate with liposomes containing MBQ-167. To quantify the amount of MBQ-167 in the liposomes, we determined the excitation/emission parameters of the molecule and measured the concentration of MBQ-167 by fluorescence. We tested the effect on cell viability of the liposomes on a Trastuzumab resistant metastatic cell line by MTT assays. We found an increase in thiol groups after the reaction with Traut's reagent, which decreased after mixing with the lipids, suggesting the formation of the DSPE-PEG-Trastuzumab conjugate. Additionally, we determined the excitation/emission parameters (320 nm/430 nm), quantified a lower limit of detection (LLOD) at 0.1 mM, and calculated an encapsulation efficiency of ≈96%. Empty liposomes showed no

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effect on cell viability, while MBQ-167-loaded liposomes decreased cell viability. Overall, our results show that our formulations efficiently encapsulate MBQ-167 and are able to deliver our compound to Trastuzumab resistant cells to reduce cell viability. Future studies include testing our formulation *in vivo* in HER2+ breast cancer models.

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Abstract 1870

Screening and characterization of allosteric small molecules that target Bruton's Tyrosine Kinase

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Bruton's Tyrosine Kinase (BTK) is a non-receptor tyrosine kinase that belongs to the TEC family of kinases. BTK contains the regulatory Pleckstrin homology-Tec homology (PHTH), Src-homology 3 (SH3), and Src-homology 2 (SH2) domains at its N-terminus followed by the C-terminal catalytic kinase domain. The expression of BTK is restricted to cells of hematopoietic lineage and is a drug target for B-cell lymphomas as these lymphomas rely on an intact B-cell receptor signaling cascade for survival. All FDA approved drugs for BTK target the ATP binding site of the kinase domain leading to adverse events due to off-target inhibition and resistance mutations occur in a subset of patients rendering the drug treatment ineffective. Therefore, allosteric sites on BTK should be exploited for drug development to combat drug resistance and target BTK more specifically. We used a combination of virtual screening, *in vitro* screening, and biochemical characterization of top hits to understand how these molecules influence BTK. To start our virtual screening campaign, we utilized a combination of DOCK blaster and Schrödinger to screen multiple virtual libraries against putative allosteric sites within BTK. Using both qualitative and quantitative measures, we narrowed our hits to ∼2000 compounds for *in vitro* screening. We used a thermal shift assay to screen our compound library against full-length BTK with Ibrutinib blocking the active site thus narrowing our interest to five compounds. We then tested these five compounds in a kinase activity assay using full-length BTK found that while all compounds enhanced BTK autophosphorylation, compound C2 activated BTK most robustly with a ∼14-fold enhancement at 250 μM C2. We determined that C2 modulates the catalytic activity of BTK but not the isolated kinase domain consist with an allosteric mode of action. This work is of interest given significant efforts to develop allosteric modulators of protein kinases and suggests that BTK can be targeted allosterically which may be exploited for further small molecule development to combat drug resistance.

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Abstract 1880

Using UV-Vis spectroscopy to determine the binding of small molecules to Peptidoglycan-Associated Lipoprotein from *E. coli*

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Peptidoglycan-Associated Lipoprotein (Pal) is a periplasmic protein that is tethered to the inner leaflet of the outer membrane of most Gram-negative bacteria. In *Escherichia coli* (*E. coli*), Pal also interacts with the peptidoglycan layer and several Tol proteins as part of the Tol-Pal complex. The Tol-Pal complex has been shown to be important for maintaining the integrity of the cell envelope and plays an integral role in cell division. As part of the complex, Pal binds to TolB, which transports Pal to the central septum during cell division. In consideration of this important protein-protein interaction, we aimed to identify small molecules that would bind Pal and disrupt the Pal-TolB interaction. Here, we describe our study, which employed UV-Vis spectroscopy to track the binding of small molecules, such as flavin mononucleotide (FMN), riboflavin, and sulfaguanidine, to Pal. Preliminary data suggest that the presence of FMN significantly affects the UV-Vis absorption spectrum of Pal (and vice-versa), suggesting that the two molecules are interacting. Other biochemical experiments are underway to corroborate these findings, with the goal of identifying a small molecule that could act as an antibiotic by inhibiting the Pal-TolB interaction, thereby disrupting cell division.

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Abstract 1901

Optimization of Pap31 Expression for E-DNA Biosensor for Carrion's Disease

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Carrion's Disease can be deadly if left untreated, and is characterized by infection with bacteria called *Bartonella bacilliformis*. This disease is common in South America, especially Peru, and is spread through both untreated water and through the Lutzomyia fly. Treatment for this disease is typically performed with either antibiotics or through proactive prevention using pesticides to reduce populations of the Lutzomyia flies. However, current detection methods for *B. bacilliformis* are often ineffective or slow, and frequently require specialized lab equipment that may not be available on-site. For these reasons, a rapid, field-portable method is necessary for earlier diagnosis and thus treatment of the disease. Pap31, a bacterial surface adhesin protein in *B. bacilliformis*, has previously been identified as a unique surface biomarker. Here, we detail the use of this protein to create a biosensor-based method to address the identified needs for Carrion's Disease. Electrochemical DNA-aptamer based (E-DNA) biosensors have been shown to be rapid, effective in complex and untreated fluids, and field portable. Previously, putative aptamers against Pap31 were identified by our lab, but validation of that aptamer requires large amounts of purified recombinant Pap31 protein. Currently, we are optimizing expression of this protein through a pET-vector based expression in *E. coli* with lactose operon induction. We will then use this protein to fully characterize which of the 6 DNA aptamers most effectively binds to and recognizes the presence of Pap31 through electrophoretic mobility shift assay (EMSA) testing. The more effective aptamer will be used to construct an E-DNA sensor that will be verified in simple and complex media with recombinant Pap31 to ensure its effectiveness as a biosensor. Ultimately, such a biosensor would enable faster, more effective testing for this disease, especially in rural areas where improved clinical outcomes are vital to saving lives.

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Abstract 1902

Electrochemical DNA-based Biosensor to Detect Pap31 Protein of Infectious Bacterium Causing Carrion's Disease

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Carrion's disease is a neglected tropical infection caused by the bacterium *Bartonella bacilliformis*. Native to South America and transmitted into human vectors via certain phlebotomine species, infection by *B. bacilliformis* includes both an acute phase of symptoms, such as fever, hemolytic anemia, and myalgia, in addition to a chronic phase, which triggers the proliferation of endothelial cells, often resulting in blindness, and/or skin lesions in the form of Peruvian warts. Indeed, the acute phase of Carrion's disease can be fatal if undetected and left untreated, making early detection of infection by *B. bacilliformis* in humans an essential effort. However, most current methods employed in the detection of Carrion's disease display low sensitivity and require lengthy, expensive workups in a well-outfitted lab setting. Electrochemical DNA-based (E-DNA) biosensors have proven to be rapid, portable, and effective in their ability to detect the presence of small molecule targets through binding to DNA aptamers specific to their target of interest. Therefore, to address the critical challenge of detection, we have selected an aptamer specific to Pap31, a protein found on the extracellular matrix of *B. bacilliformis*. This aptamer is being adapted to a biosensor format via guided truncation to a minimally active aptamer, then incorporation into a DNA structure that will change conformation upon binding Pap31. This conformation change is turned into an electrochemical voltammetric readout due to the incorporation of methylene blue, a redox-active tag. This will allow the transduction of Pap31 binding into an electrical current signal, and we are verifying the sensitivity and specificity of this biosensor approach. The successful development of an E-DNA biosensor that is fast, effective, and portable for the detection of Carrion's disease would offer new possibilities for treatment and health outcomes in many South American communities.

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Abstract 1907

Delivery of Apolipoprotein E3 Reconstituted High-Density Lipoprotein Nanodiscs for the Treatment of Lysosomal Storage Diseases

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Lysosomal storage diseases (LSDs) are inherited metabolic diseases that are due to enzyme deficiencies which affect the function of lysosomes. Niemann-Pick disease types A and B are LSDs that are caused by the deficient enzyme, acid sphingomyelinase (ASM), resulting in the toxic buildup of sphingomyelin. Despite potential treatments such as enzyme replacement therapy (ERT), ERT lacks the ability to cross the blood-brain barrier for eventual delivery of recombinant enzymes to the lysosomes. Alternatively, developing a reconstituted high-density lipoprotein (rHDL) nanodisc surrounded by apolipoprotein E3 (apoE3) and recombinant ASM (rASM) may prove effective in targeting lysosomes. rHDL is an ideal platform for the delivery of enzymes while apoE3 is known to bind low-density lipoprotein receptors (LDLr) and can facilitate receptor-mediated endocytosis (RME). Our overall objective is to determine whether the fusion of the apoE3 receptor-binding segment with functional ASM can bind to LDLr and be targeted by RME to the lysosomes. We have designed a plasmid construct consisting of the apoE3 N-terminal domain and the ASM C-terminal domain, linked by a flexible linker, with a hexa-His-tag attached at the N-terminal end. ASM-apoE3NT was overexpressed, isolated, and purified in *E. coli* by affinity chromatography. In preliminary studies, we have generated rHDL nanodiscs by reconstitution of 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and ASM-apoE3NT by incubation, followed by density gradient ultracentrifugation. In the next steps, we will carry out structural characterization of ASM-apoE3NT, assess enzyme activity, and determine cellular uptake and lysosomal localization. Successful findings from this study may provide a non-invasive and blood-brain barrier-targeted procedure for the delivery of large molecule therapeutic agents to treat LSDs.

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Abstract 1929

A simplified workflow for plasmid transformation of and protein expression in *Vibrio natriegens* (VmaxTM X2)

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E. coli has been the workhorse for molecular cloning and protein expression for decades; however, *Vibrio natriegens* is rapidly emerging as a potential replacement for *E. coli* for these functions. *Vibrio natriegens* has a doubling speed twice that of *E. coli*, which could be advantageous during recombinant protein production. A recently developed, commercially available strain of *Vibrio natriegens* (VmaxTM X2) regulates protein expression under the familiar IPTG-inducible T7 promoter system. In further simplifying the protein production workflow, the transformation of VmaxTM X2 cells can be accomplished without electroporation or heat shock via modification with the Mix&Go!TM chemically competent kit. Standard and treated VmaxTM X2 cells were grown pre-transformation and post-transformation in a variety of media and at two temperatures and then compared to BL21(DE3) *E. coli* pre- and post-transformation. Both strains were transformed with three plasmids and protein expression was measured by western blot and SDS-PAGE. Eliminating the heat shock and electroporation stage of transformation increasing the potential use of the VmaxTM X2 cells by simplifying the protein production process.

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Abstract 1941

Approaches to produce functional recombinant human serine protease PRSS23 catalytic domain

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Serine Protease 23 (PRSS23) is a little-studied putative serine protease. It is predicted to be an active serine protease based on sequence homology, including the conservation of the amino acid residues that define the serine protease catalytic triad. A few studies have shown correlations between PRSS23 gene expression and several diseases. Furthermore, preliminary data from our lab indicate that PRSS23 expression is correlated with poor outcomes in ovarian cancer, and that PRSS23 expression promotes increased anoikis resistance and proliferation of ovarian cancer cells. These observations suggest that PRSS23 may offer a novel therapeutic target; however, its targeting would require knowledge of the protein structure and function as an enzyme that are not yet available. Here, we aim to generate recombinant PRSS23 in its native or folded form to enable studies that will define PRSS23 proteolytic activity, structure, and functional role in disease progression. Despite extensive optimization efforts, our attempts to express the full-length protein in HEK 293 Freestyle cells and Pichia pastoris gave barely detectable yields. The catalytic domain could be well-expressed in *E. coli*, but was found exclusively in insoluble inclusion bodies. Next, we devised two strategies to attempt recovery of the folded PRSS23 catalytic domain: (a) extracting the protein from inclusion bodies and refolding it *in vitro*, and (b) expressing soluble, folded protein from *E. coli* through fusion to the maltose binding protein (MBP), a highly soluble protein that can act as a molecular chaperone. To refold the protein *in vitro*, we first extracted the His-tagged PRSS23 catalytic domain from inclusion bodies, purified it by nickel affinity chromatography under denaturing conditions, and then tested various refolding buffers. In small-scale trials, we identified conditions to successfully refold the recombinant protein as assessed by particle size measured via Dynamic Light Scattering (DLS). Current efforts focus on scale-up of the refolding protocol and identification of storage buffers that stabilize the folded state. In parallel with refolding efforts, we expressed the His-tagged MBP-PRSS23 fusion protein in the periplasm of *E. coli*, and purified the fusion protein by nickel and amylose affinity chromatography. Current efforts are focused on optimizing full-length fusion protein production, further purification to homogeneity, and *in vitro* cleavage of the fusion linker to release the pure PRSS23 catalytic domain. Once pure preparations of soluble recombinant PRSS23 catalytic domain are obtained by either or both approaches, we intend to test various potential substrates to define PRSS23 proteolytic activity. We also plan to crystallize the protein for structural studies and to determine its effects on ovarian cancer cells.

ASBMB

Ultimately, we anticipate that our studies of recombinant PRSS23 will define the structure and biochemical function of a novel enzyme, providing key information to enable its potential targeting in disease.

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Abstract 1950

Diagnostic application of dCas9 and graphene oxide for rapid and sensitive detection of antibiotic resistant genes

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The rapid detection of an infection with an antibiotic resistant pathogen is key in maintaining public health, especially with the continuous rise of such pathogens. The current gold standard for the detection of these infections is through a technique called polymerase chain reaction which is a sensitive detection method, yet rather expensive, laborious, and time-consuming which is not ideal for fighting such infections. To combat this, we designed a rapid yet sensitive assay using deactivated Cas9 protein (dCas9) and graphene oxide (GO). dCas9 utilizes complementary base pairing to bind specific double-stranded DNA with single-guided RNA (sgRNA). For this project, the sgRNA is designed to target a tetracycline resistant gene (tetM) and is labeled with a fluorescent molecule, fluorescein. The dCas9 and labeled sgRNA are complexed and then incubated with GO for 15 minutes in an assay where the GO and dCas9 can coalesce through electrostatic interactions. Graphene oxide is a 2-D nanosheet that is able to quench fluorescence. When the dCas9 is adsorbed onto the GO, the fluorescence from the labeled sgRNA is quenched. However, when target DNA is introduced and binds to the dCas9, the protein-sgRNA complex will undergo a conformational change, causing the dCas9 to release from the GO surface, thus restoring the labeled sgRNA's fluorescence. This restoration of fluorescence can then be quantitatively measured to determine if the target DNA is present within a sample. Our approach was able to achieve sensitive detection of DNA down to the concentration of 10 pM only after a 15-minute incubation. Future studies will focus on even lower concentrations and determine its specificity.

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Abstract 1955

Intracellular SERS detection of nanoparticle drug delivery platforms

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Numerous multifunctional nanomaterials have been designed and tested as anti-cancer agents, bringing to focus a set of challenges such as system stability, localization, biodistribution, biocompatibility and efficiency. As a solution to these challenges, we proposed a nano-system composed of Graphene Quantum Dots (GQDs) covering a Gold-Silver (AuAg) alloy core to aid in anti-cancer therapy and imaging. GQDs belong to carbon based nanomaterials, known to be efficient drug delivery platforms with high biocompatibility. Applications for alloy nano-systems include being substrates for surface-enhanced Raman spectroscopy (SERS) and colorimetric detection, both serving for early cancer detection as well as drug delivery tracking. Because of these properties and applications, this project explores GQD-covered AuAg nanoparticles and lone GQD systems as a promising alternative to tackle present cancer treatment, drug delivery and diagnosis challenges. In this study, nanomaterial cell internalization was detected via a SERS substrate, using prostate cancer cells (PC-3) as a model. The results obtained gave a signal strong enough to identify the nanomaterial it was incubated with.

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Abstract 1957

The Building of Protein-DNA Scaffold Co-Crystals

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X-ray crystallography is a common technique utilized in the field of molecular biology for the determination of structure at the atomic scale. It functions by observing an X-ray beam diffract through a crystal structure; the angles and intensities of which can then be used to recreate the 3-D geometry and electron density within the crystal. This has the unfortunate requirement of needing a well-ordered crystalline structure with relatively immobile molecules within it. In the context of solving protein structures, this requires sampling many conditions to identify ones that are suitable for crystal growth; a tedious process that can take years to accomplish. The goal of this project is to use isoreticular co-crystals to build a modular scaffold crystal consisting of protein and DNA for use in scaffold-assisted x-ray crystallography. Proteins containing either natural or engineered DNA binding sites could then be loaded into these porous crystals and imaged using X-ray crystallography without the long process of crystallization. These crystals are stabilized by stacked DNA as well as protein-protein interfaces. Previous work has succeeded in growing stable crystals with small pores; however, the size of these pores is limiting to the size and success of guest installation. Further work has succeeded in growing co-crystals with increased solvent-accessible pores through the insertion of a short expander sequence. However, these crystals demonstrate greatly increased fragility, prompting investigation into methods of stabilization. One strategy for stabilizing these crystals is engineering of the protein-protein interface to support form an intermolecular-protein disulfide bonds. These bonds are hypothesized to form spontaneously when the crystal is under oxidizing conditions and would stabilize the crystal at the protein-protein interface. At present, three mutants have been designed, engineered, and expressed in *E. coli*. In the near future, the presence of each an additional, surface-accessible engineered cysteine will be confirmed through an Ellman's Reagent Assay and we will begin screening conditions for optimal crystal growth. If successful, this work would allow for the easy and reproducible growth of self-crosslinking scaffold crystals that can be used for the installation of any small-to-medium-sized, ordered protein containing a DNA binding domain. Ideally, subsequent imaging via X-ray crystallography will result in high-throughput, high-resolution structure determination.

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Abstract 1964

Axolotl: On your MARCKS, ge

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The Axolotl (Ambystoma mexicanum) has puzzled scientists for many years but with the discovery of a new protein, we might be one step closer to discovering the key to its limb regeneration. The formation of a blastema, a mass of multipotent progenitor cells that can differentiate into novel limb structures, is a crucial step in limb regeneration. Scientists have identified the Myristoylated Alanine-Rich C-kinase Substrate (MARCKS), a ubiquitous protein that is involved in neural regeneration and other body parts; and MARCKS-like Proteins (MLP), a molecule that shares homogeneous and functional similarity with MARCKS, as potential factors for regeneration, although the specific regenerative mechanisms of the axolotl remain elusive. Both proteins are prevalent molecules that can be phosphorylated by Protein Kinase C (PKC). Upon phosphorylation, the direct interactions between MLPs and the cytoskeleton regulates cell migration. Recently, a study first identified that axolotl MLPs (AxMLPs) are the wound-associated molecule that initiates the regeneration process by inducing cell cycle activities. The effector domain (ED) within the 224 amino acids of the MLP is a domain with high affinity for phosphatidylinositol 4,5-bisphosphate (PIP2). This allows for the interaction between MLP and this membrane component, which is involved in numerous cellular functions including cytoskeletal actin dynamics, membrane trafficking, cell migration, and mitosis. The MLP inside axolotls is like that found in humans, with percent similarities of 74.1%, 68.0% and 80.0% in its three domains. The resemblance between blastema and human stem cells could provide insight into research on regenerative medicine but may bring about ethical concerns. The Ashbury College Center for BioMolecular Modeling A Protein Story (MAPS) team used 3D modeling and printing technology to examine the structure-function relation of the MARCKS-like Protein and its contribution to axolotl limb regeneration.

The Ashbury College Center for BioMolecular Modeling A Protein Story (MAPS) team used 3D modeling and printing technology to examine the structure-function relation of the MARCKS-like Protein and its contribution to axolotl limb regeneration.

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Abstract 1965

Characterization of the Uptake of Small Extracellular Vesicles Derived from Cow's Milk in the Gastrointestinal Tract

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Jane Jourdan, Spencer Marsh, Robert Gourdie

Background: Small extracellular vesicles (sEVs) can be loaded with drugs, allowing for the oral delivery of therapeutics generally administered via a subcutaneous or intravenous route. Engineering vehicles, such as sEVs, for the delivery of fragile drugs orally can be challenging and costly, however, bovine milk sEVs may offer an inexpensive and scalable solution to this problem. Currently, the mechanism of bovine milk sEV uptake in the human gastrointestinal tract is not known; however, recent research has demonstrated that an interaction between bovine milk sEV-IgG and the neonatal Fc receptor (FcRn) on intestinal epithelium may be significant. Understanding the process by which bovine milk sEVs are absorbed would allow us to modulate the pharmacokinetics of this promising new vehicle for drug delivery.

Objective: Is IgG important for bovine milk sEV uptake in the gut? We hypothesize that bovine milk sEVs are taken up by intestinal epithelial cells via an interaction between bovine milk sEV-bound IgG and the neonatal Fc receptor (FcRn).

Methods: Bovine milk sEVs were analyzed via dot blot and 260/280 spectrophotometry for IgG and protein concentration, respectively. To investigate the role of sEV-bound IgG on the uptake of bovine milk sEVs in the gastrointestinal tract, a Caco-2 cell model was used. The FcRn on Caco-2 cells and the IgG on bovine milk sEVs were blocked using bovine IgG and Protein G, respectively. Caco-2 cells were incubated with fluorescent bovine milk sEVs and analyzed for immunofluorescence. Milk sEV uptake into Caco-2 cells was quantified using ImageJ software. To account for FcRn recycling at the membrane, the FcRn block was applied at 0, 30, and 120 minutes prior to bovine milk sEV incubation. Controls were cells and bovine milk sEVs without a block and sEVs without a fluorescent marker.

Results: Linear regression analysis revealed that bovine milk sEVs have 1.84 ng of IgG per mg sEV. Immunofluorescence revealed that FcRn is present on Caco-2 cells with some receptors present on the cell membrane. Immunofluorescence also revealed that bovine milk sEVs are taken up by Caco-2 cells regardless of whether the FcRn-sEV-IgG interaction was blocked ($P > 0.5$). Additionally, pre-treating Caco-2 cells with IgG increased the amount of bovine milk sEV uptake ($P < 0.05$).

Conclusions and Future Directions: *In vitro* assays designed to block the bovine milk sEV-IgG-FcRn interaction did not decrease bovine milk sEV uptake into Caco-2 cells. However, pre-treating Caco-2 cells with IgG yielded increased bovine milk sEV uptake into cells, suggesting a relationship between these proteins may impact bovine milk sEV uptake in

the gut. Future work includes analysis of possible co-localization of fluorescent labeled-IgG and bovine milk sEVs, experiments at acidic pH, and trans-well assays on differentiated Caco-2 cells.

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Abstract 1980

Characterizing metabolic drivers of *Clostridioides difficile*

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Many enzymes require post translationally installed cofactors for function that are highly regulated and allow for monitoring of metabolic activity. *Clostridioides difficile* is a gut anaerobe that causes severe infection of the colon mostly associated with antibiotic induced dysbiosis. During colonization and infection, *C. difficile* is dependent on amino acid Stickland fermentation over central carbon metabolism, suggesting fermentation of amino acids sustains *C. difficile* during disease progression. Here using reverse-polarity activity-based protein profiling (RP-ABPP), we revealed Stickland enzyme activity is a biomarker for *C. difficile* infection (CDI) and annotated proline reductase and glcyine reductase, two metabolic cofactor-dependent Stickland reductases. We structurally assigned these reductases as cysteine-derived pyruvoyl-dependent enzymes through chemical proteomics and showed through cofactor monitoring that their activity, similar to their expression, is regulated by their respective amino acid substrates while additionally demonstrating their interdependent regulation. Proline reductase was found consistently active in toxigenic *C. difficile* laboratory strains and clinical isolates from pediatric cases of CDI (deidentified; IRB approval and collection in previous work. No human patients were used in this work), confirming the enzyme to be a major metabolic driver of CDI. Further, through direct cofactor targeting and observed by fluorescence-based enzymatic assays, activity-based hydrazine probes were shown to be active site directed inhibitors of proline reductase Therefore, we suggest proline reductase activity is a promising therapeutic target that could allow for resurgence of the microbiota that compete with *C. difficile* for proline and subsequent restoration of colonization resistance against *C. difficile* in the gut.

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Abstract 1990

Receptor-mediated STING agonist delivery by mannose-decorated lipoprotein nanoparticles can repolarize ovarian cancer-educated macrophages

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Cancer cell-derived factors in epithelial ovarian cancer (EOC) can polarize tumor-associated macrophages (TAMs) to an immunosuppressive (M2) phenotype. The M2-TAMs, in turn, contribute to cancer cell proliferation, metastasis as well as to the immunosuppression, and drug resistance often reported in EOC patients and EOC murine models. Hence, targeting TAMs in EOC and promoting an immunostimulatory (M1) phenotype in the tumor microenvironment (TME) can be beneficial in anticancer therapy as TAMs are heavily represented in TME of EOC and mediate the interplay between the immune system and the tumor. The ubiquitous presence of macrophages in the body, the low bioavailability of immunomodulatory agents, and the complex architecture of the TME pose a challenge to the selective targeting of TAMs in EOC. In this study, we evaluated mannose-decorated reconstituted high-density lipoproteins nanoparticles (mrHDL NPs) as a delivery vehicle to macrophages exposed to conditioned media from EOC cells. M2-TAMs often show high expression of the mannose receptor (CD206). HDL particles have natural carrier capabilities and can interact with macrophages via the HDL receptor. We hypothesized that the mrHDL NPs can be efficiently loaded with a hydrophobic cargo (drug or dye) and will subsequently deliver the cargo to EOC conditioned media-educated macrophages via the HDL receptor. DMXAA, an agonist of the murine stimulator of interferon genes (mSTING), was used as a model drug cargo. The loading of DMXAA in the mrHDL NPs was assessed via absorbance and fluorescence measurements. RAW 264.7 mouse macrophages were incubated with the mouse EOC cell line ID8-conditioned media (ID8 CM) for 24 hours to simulate an *in vitro* model of TAMs. An increase in fluorescence anisotropy of DMXAA incorporated into mrHDL NPs compared to free DMXAA indicated that the DMXAA was loaded into mrHDL NPs. Western blot analysis and enzyme-linked immunosorbent assays (ELISA) showed that the ID8 CM-treated macrophages expressed the HDL receptor, CD206 as well as other immunosuppressive markers and cytokines. Similar to the free DMXAA, the mrHDL-DMXAA NPs elicited secretion of immunostimulatory cytokines including IFN-β and CXCL10 from ID8 CM-treated macrophages. Inhibition of the HDL receptor and blocking the mannose receptor reduced dye uptake in ID8 CM-treated macrophages from mrHDL-dye NPs. These findings confirm our hypotheses and suggest that not only the HDL receptor, but also, the mannose receptor mediates the uptake of the cargo from the mrHDL NPs. This mode of cargo delivery can help circumvent challenges associated with utilizing TAM-targeting agents, including STING agonists, and provides the mrHDL NPs with the potential for clinical application as a delivery vehicle for TAMs in EOC.

This study was partially supported a grant (#RP210046) from the Cancer Prevention and Research Institute of Texas (CPRIT) (A.S.D.) and by the National Heart Lung and Blood Institute (NHLBI) of the National Institutes of Health Award #5R25HL007786-29 (M.M.). This study also was partly funded by the Virginia Morris Kincaid Cancer Foundation.

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Abstract 1998

Pharmacological activities of *Sinningia bullata* extracts

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Cheng-Yang Huang

Sinningia bullata is a tuberous member of the flowering plant family Gesneriaceae. Prior to this work, the antibacterial, antioxidant, and cytotoxic properties of *S. bullata* were undetermined. Here, we prepared different extracts from leaves, stems, and tubers of *S. bullata* and investigated their pharmacological activities. The leaves extract of *S. bullata*, obtained by 100% acetone (SB-L-A), had the highest total phenolic content, total flavonoid content, cytotoxic effects, antioxidation capacity, and antibacterial activities. SB-L-A displayed broad range of antibacterial activities against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The inhibition zones of SB-L-A ranged from 8 to 20 mm and were in the following order: *S. aureus* > *E. coli* > *P. aeruginosa*. Incubation of B16F10 melanoma cells with SB-L-A at a concentration of 80 μg/mL caused deaths at the rate of 96%, reduced migration by 100%, suppressed proliferation and colony formation by 100%, and induced apoptosis, which was observed in 96% of the B16F10 cells. SB-L-A of 80 μg/mL boosted the distribution of the G2 phase from 6.0% to 26.4% in the B16F10. Accordingly, SB-L-A might suppress melanoma cell proliferation by inducing G2 cell-cycle arrest. The most abundant compounds in SB-L-A were also identified using gas chromatography–mass spectrometry to assess whether some active ingredients present in SB-L-A are useful alone or in combination by exerting cytotoxic effects on melanoma cells. Overall, the collective data in this study indicate the pharmacological potential of SB-L-A for further medical applications.

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Abstract 2005

The Interplay Between Strong and Sustained aUPR Activation, Necrosis, and Autophagy

Florina Gojcaj, *University of Detroit Mercy*

Mara Livezey, Destiny Proffett

Recurrent estrogen receptor α (Era)-positive breast and ovarian cancers are often therapy resistant. The molecule 1,3-Dihydro-3,3-bis(4-hydroxyphenyl)-7-methyl-2H-indol-2-one (BHPI), selectively blocks the proliferation of these therapy resistant cancer cells. BHPI is a biomodulator of ERa that activates the anticipatory unfolded protein response (aUPR). The aUPR is normally protective; it prepares cells for the additional protein folding load expected upon proliferation. BHPI hijacks this protective activation of the aUPR, and causes strong, sustained, and eventual cytotoxic activation. It was previously shown that BHPI induces necrotic cell death through initiation of a futile cycle of calcium leaking and pumping in the endoplasmic reticulum. This futile cycle eventually leads to ATP depletion within cells. In addition to inducing ATP depletion and necrotic cell death, BHPI has been shown to inhibit cellular autophagy. We are looking to understand if inhibition of cellular autophagy plays a role in the initiation of ATP depletion and necrotic cell death. Autophagy marker proteins, such as Beclin-1 and LC3 were targeted using western blotting. Follow-on assays were then used to further clarify the interplay between ATP depletion, autophagy, and necrosis.

I would like to acknowledge the University of Detroit Mercy for providing the facilities for us to meet and do research in. I would also like to thank the faculty research awards through the University of Detroit Mercy and the National Institutes of Health ReBUILDetroit grant 5RL5GM11898 that fund laboratory supplies, allowing us to conduct new experiments.

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Abstract 2010

Graphene Quantum Dots- covered AuAg alloy nanoparticles as Drug Delivery Platforms for Anticancer Applications

Alondra Veloz, *University of Puerto Rico, Rio Piedras*

Gerardo Morell, Brad Weiner, Nataniel Medina

Prostate cancer is a form of cancer that develops in the prostate gland, a small walnut-shaped gland in the pelvis of men. It is also the second-leading cause of cancer deaths for men in the U.S. Treatment using chemotherapy affects our cancer cells and our healthy cells, causing many side effects such as hair loss, vomiting, and pale skin. Because of this, chemotherapeutic drug delivery based on nanotechnologies has been proposed. Many potential drugs that can overcome chemotherapy's side effects are limited due to their poor water solubility. This study is focused particularly on noble metal alloy nanoparticles, such as silver and gold, covered in graphene quantum dots to improve the solubility and bioavailability of hydrophobic drugs with potential for chemotherapy. Gold and silver alloy nanoparticles have been shown to induce significant apoptosis and cell necrosis, in various cell line studies and have been proposed as nontoxic carriers for drug and gene-delivery application. Graphene quantum dots serve as a fluorescent layer to prevent the oxidation of the metal nanoparticles, and at the same time enabling drug delivery tracking. To identify the 50% of the concentration (IC50) that will kill the cell, we validate the IC50 with viability tests in prostate cancer (PC-3) cells. Another technique used was the confocal microscopy where we were able to see where the drug delivery systems were located and through how much time in the cell, where the microscope combines images from different levels of the sample to form a 3D image of the cells studied. The nanoparticles' interactions with the blood were also studied to understand how it changed the morphology of the blood cells. Our goal is to demonstrate that the nanoparticles we have do not have harmful interactions with red blood cells and to determine their toxicity towards PC-3 cells.

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Abstract 2013

Purification of Rubredoxin tagged Recombinant Proteins using the Three Phase Partitioning technique

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Musaab Al-Ameer, Patience Okoto, Paul Adams, Suresh Thallapuranam

Challenges remain in the production and purification of recombinant proteins and peptides. Conventional Chromatography usually involves multiple steps and often requires specialized equipment and a significant investment of time and labor. Herein, we aim to overcome some of these concerns by utilizing rubredoxin (RD), a 7 kDa hyperthermophile protein from Pyrococcus furiosus. RD enhances the recombinant peptide expression preventing the formation of inclusion bodies. It possesses red color that aids in the detection and quantification of target proteins. Furthermore, we have successfully incorporated a three-phase partitioning (TPP) technique which involves the addition of ammonium sulfate and tert-butanol to the crude extract of RD-peptide forming three layers. We modified our TPP method by adding a high concentration of urea to isolate and concentrate Rd-fused peptides in the urea layer while cellular debris salts out in the middle layer. Using this strategy, we purified Rd-fused recombinant proteins from a cell lysate in less than 30 minutes. Results highlight crucial information to develop an affordable, Time-efficient, and easy-to-use purification scheme for recombinant proteins/peptides.

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Abstract 2015

Evaluating effect of metalloproteinases and their natural inhibitors on blood brain barrier

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Hannaneh Ahmadighadiekolaie, Janet Lambert, Arman Hosseini, Masoud Kalantar

Metalloproteinases (MPs) play key physiological and pathological roles in the central nervous system by regulating signaling pathways during neuroinflammation, blood brain barrier (BBB) disruption, synaptic dysfunction, or neuronal death. Upregulation of specific MPs contributes to Alzheimer's disease (AD) pathology as some of the metzincin family, such as MMP-9 is associated with synaptopathic neurodegenerative disorders. MMP-9 is upregulated in the brain tissue of AD patients. Overexpressed MMP-9 has been found in the cytoplasm of neurons, neurofibrillary tangles, senile plaques, and vascular walls of the hippocampus and cerebral cortex of AD patients, and inhibiting MMP-9 improves Aβ-mediated cognitive impairment and neurotoxicity in mice. We have used an *in vitro* BBB model by growing rat brain microvascular endothelial cells on 24-well transwell inserts. We showed that MMP-3cd recombinant protein disrupts the integrity of BBB measured by permeability assay using a fluorescein isothiocyanate dextran. We also performed trans endothelial electrical resistance (TEER) to measure resistance of BBB which indicates formation or disruption of tight junctions between endothelial cells. The results showed MMP-3 increased permeability of BBB in a dose-dependent manner, while inhibition of MMP-3 using TIMPs resulted in protection of BBB. We are also evaluating effect of TIMP variants on protection of BBB. We expect that this study will result in potential therapeutics for neurodegenerative diseases which MMPs play a critical role.

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Abstract 2017

Conformation-dependent Inhibition of the AML-associated Src-family kinase Fgr by ATP-site inhibitors

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Shoucheng Du, John Alvarado, Thomas Smithgall

Development of acute myeloid leukemia (AML) is often associated with overexpression of non-receptor protein-tyrosine kinases, including three members of the Src family: Hck, Lyn and Fgr. Our group is actively investigating small molecule Src-family kinase inhibitors with significant anti-AML efficacy. These inhibitors include the pyrrolopyrimidine A-419259 and the N-phenylbenzamide TL02-59; the latter compound potently inhibits Fgr and Lyn *in vitro* and reverses bone marrow engraftment of the human AML cell line MV4-11 in a mouse model. We recently solved X-ray crystal structures of near-full-length Fgr, consisting of the SH3, SH2, and kinase domains plus the tyrosine-phosphorylated tail, in complex with each inhibitor. A-419259 bound to the Fgr ATP-site with the regulatory SH3 and SH2 domains packed against the back of the kinase domain, resulting in a closed conformation observed in previous structures of Hck with this inhibitor. However, while TL02-59 also bound to the Fgr ATP-site, it induced allosteric displacement of the SH3 and SH2 domains from their regulatory positions, resulting in an open conformation. To explore the effect of allosteric domain displacement on the TL02-59 inhibitory mechanism, Fgr mutants were generated with enhanced SH3 domain interaction with the SH2-kinase linker (high affinity linker or 'HAL' mutants). Fluorescence polarization assays confirmed enhanced intramolecular SH3: linker interaction, thus favoring the closed conformation. X-ray crystallography of an Fgr SH3-SH2-linker protein with the HAL substitution showed that the orientation of the SH3 and SH2 domains is virtually identical to that observed in the structure of near-full-length Fgr bound to A-419259, suggesting that the high affinity linker does not impact the overall closed kinase conformation. To test the effect of HAL substitutions on Fgr sensitivity to TL02-59, we created an active form of Fgr by fusing it to the coiled-coil (CC) domain of the breakpoint cluster region protein (Bcr). Expression of CC-Fgr (wild-type and HAL mutants) transformed TF-1 cells into a cytokine independent phenotype and rendered them sensitive to Fgr inhibition by TL02-59. Stabilizing the closed conformation by introduction of the HAL mutations enhanced TL02-59 potency in these cells, suggesting that TL02-59 prefers a single Fgr kinase conformation. Ongoing work is directed toward identification of small molecules that mimic the effect of HAL substitutions on Fgr. Combining allosteric modulators that lock a single Fgr conformation are predicted to synergize with TL02-59 and suppress the evolution of resistance mutations, a

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common limitation of many clinical ATP-site kinase inhibitors currently in use for AML and other cancers.

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Abstract 2019

Quantum Electrochemical Spectroscopy (QES), a broad bioanalytical platform for robust and scalable data collection from biological systems

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The increasing availability of biological and biomedical data, such as genomic information, has set the stage for the development of artificial intelligence (AI) solutions that capture the complexity of biology. However, while genetics provides the blueprint, proteins, metabolites, and other molecules are the effectors of biology. There are more than 1 000 000 proteoforms and 200 000 metabolites, fluctuating in form and abundance in response to environmental, health, and lifestyle changes in the human body. Measuring this complexity at scale requires undesired tradeoffs. Simpler and targeted techniques (e.g., immunoassays) limit the breadth of the analysis, while complex technologies (e.g., LC-MS) provide a broader view but at the cost of limited datasets and statistical power in the study. To enable AI to impact biological research and, eventually, healthcare, we need an approach that breaks the tradeoffs and incrementalism of existing analytical tools and produces reliable multi-dimensional data at scale. We describe here a novel spectroscopy-like technique, Quantum Electrochemical Spectroscopy (QES), for the scalable generation of vibrational signatures of molecular species in biological samples. The QES analytical approach leverages a simplified, sample-preparation-free workflow, with a minimal sample consumption (2–4 ul), to generate high dimensional vibrational signatures. Machine-learning-derived digital filters are applied to these signatures to deconvolute sample biochemistry into expression profiles of specific phenotypes or biomarkers associated with the sample. The QES method relies on a nanoscale electrochemical interface to transduce vibrational frequencies of biochemical species into the charge transfer current data that is acquired using custom-built low-noise instrumentation. A voltage scan and concomitant current measurement at the nano-electrochemical interface create an ensemble representation of the sample biochemistry in terms of the transduced vibrational modes. This ensemble is archived as the sample digital twin on the cloud. A set of reference samples are also created and analyzed using the QES approach to create a ground-truth representation of target phenotype or biomarker signatures, which are used to train computational classifiers to identify and isolate target-specific features in the sample vibrational signature dataset. As a result, and in contrast to traditional analytical approaches, QES decouples the measurement of the sample from the data analysis and analyte selection, creating an iterative and efficient hypothesis-generating process. Data is acquired from multiple samples in parallel with one measurement workflow. The sample analysis is

digitally customized using target-specific reference data and classifiers after the measurement process without needing specific reagents. In this study, we aim to demonstrate how QES can detect, in a multiplexed manner, analytes at multiple scales of biology, from metabolites to proteins and single cells. We also present the ability of the QES platform to distinguish between mass isotopes, structural and optical isomers, thereby enabling a new sensing modality for analyte identification, quantification, and disease-specific signature discovery.

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Abstract 2029

Narrowing down the path to identify a specific organelle that future curative therapeutic strategies for HBV could target in the cccDNA biogenesis to potentially degrade and eliminate the formation of cccDNA by rcDNA through molecular mechanisms of bioche

Victor Mudenda[1,2], *[1]Lily Flower of the Valley Corporation, Alameda, California, USA; [2]Gideon Robert University*

Goal of the Study: The goal of this study was to narrow down a path in identification of a specific organelle that future curative therapeutic strategies for HBV could target in the cccDNA biogenesis to potentially destroy and block the formation of cccDNA from rcDNA by focusing on molecular mechanisms of rcDNA – cccDNA biochemical repair process. Hepatitis B is a liver infection caused by the Hepatitis B Virus (HBV), a virus which is a partially double stranded enveloped DNA that causes hepatocellular degradation and infection and leads to liver cirrhosis as well as hepatocellular carcinoma. It is transmitted through sexual contact, from mother to baby at birth, blood and semen transmitted by an infected HBV person. It has become a global life-threatening disease that creates chronic infections in infected hepatocytes thereby putting the vulnerable population at high risk of dying from liver cancer or cirrhosis diseases. Chronic Hepatitis B (CHB) infection continues to be a major health burden worldwide. It is estimated that more than 296 million people are infected with hepatitis and nearly one million chronically infected people die from HBV annually. Currently, the HBV is not curable thereby resulting in chronicity of the disease. However, the molecular processes governing the HBV persistence and stability of the HBV genome in the hepatocyte even in the presence of current antiviral therapeutic regimes are not fully understood. Continuous formation of cccDNA from rcDNA makes HBV stable, persist and replicate in the infected hepatocytes. This study will summarize the portions in the cccDNA biogenesis that can be targeted by future curative therapies. The havoc wreaked by HBV in the infected liver. eradication of cccDNA persistence and stability in the dividing hepatocyte. The findings of my paper will highlights a detailed mechanistic view of how HBV rcDNA is repaired to form cccDNA in biochemical repair so as to lead the scientists direct their efforts on developing a curative therapeutic regime to eliminate the precursor of cccDNA and rcDNA, leaving the cells free of HBV.

Methods: A review of articles regarding the stability, persistence of HBV and unique replication strategy in the infected hepatocytes. The structure of rcDNA, cccDNA and activities of different enzymes supporting biochemical repairing were examined using In Situ methods.

Results: After a detailed review of the articles and activities shown by the In Situ methods, it was revealed that cccDNA biogenesis requires removal of the viral polymerase (POL) generating protein-free rcDNA (PF-rcDNA). The DNA flap and

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RNA primer should be removed and the single-stranded DNA gap in the (+) strand repaired to obtain the cccDNA molecule. A comprehensive list of the enzymes from the host-repair system possibly involved in cccDNA biogenesis may be identified in each phase.

Conclusion: The creation, stability and persistence of HBV covalently closed circular DNA (cccDNA) is the underlying major factor that causes HBV chronicity. However, the comprehensive molecular mechanism of cccDNA formation from relaxed circular DNA (rcDNA) remains a puzzle to solve in discovering a true curative therapeutic therapy for HBV. Only by deleting/degradation or lethal mutating the precursor of cccDNA and rcDNA can a complete cure of HBV happen. This knowledge is important because it will help create such a curative therapeutic model for HBV by which scientists may find it beneficial combination attribute to other existing therapeutic regimes to cure HBV. Thus, rcDNA conversion to cccDNA is a specific path to target.

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Abstract 2070

Determination of the importance of disubstituted-isoxazole regiochemistry in a thiourea inhibitor of Varicella-zoster virus portal protein

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Tionna Burner, Robert Visalli, David Goode

The Varicella-zoster virus (VZV) is a pathogen that causes chickenpox in children and its reactivation in adults results in herpes zoster, also known as shingles. In recent years, an anti-zoster thiourea compound has been discovered which inhibits infection from the virus. The compound targets the VZV portal protein and selectively inhibits viral replication. Through targeting viral replication, a human cell will remain virtually unaffected while preventing further infection. Previously, a disubstituted isoxazole containing thiourea showed promising inhibition in viral replication inhibition assays. However, resynthesis of the compound with the suspected 3,4-disubstituted isoxazole regiochemistry led to cellular toxicity in the assay. Thus, a synthetic pathway for the 3,5-disubstituted isoxazole was designed and is currently under optimization. Briefly, the synthetic pathway begins with formation of a chalcone with appropriate substituents on the aromatic rings through a Claisen-Schmidt condensation. Conversion of the chalcone product to an oxime followed by oxidative cyclization produces the desired 3,5-disubstituted isoxazole. Finally, reduction of a nitro substituent followed by thiourea formation produces the desired isoxazole containing thiourea compound. Synthesis through the cyclization has been achieved successfully, but optimization of the oxidative cyclization and nitro reduction steps is currently underway. Upon successful synthesis, the compound's cytotoxicity and viral inhibition ability will be compared to the 3,4-disubstituted isoxazole thiourea.

The Mercer University Chemistry Department.

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Abstract 2071

Design and synthetic pathway optimization of a thiourea compound library as potential inhibitors of Herpesviridae portal protein

Lily Dwyer, *Mercer University*

Parker Hilliard, Robert Visalli, David Goode

Herpesviridae carry a structurally conserved portal protein used for DNA packaging during viral replication. Previous studies have shown that a class of thiourea compounds show potential inhibitory effects on this process by interacting with the portal protein. To further understand the mechanism of inhibition and understand the portal protein function more clearly, a library of thiourea compounds with a phenylenediamine core were designed as potential inhibitors. The library varies in the aniline and carboxylic acid components chosen to be linked to the phenylenediamine core. The synthetic pathway to these compounds is being optimized, but currently begins with reaction between an isothiocyanate phenylenediamine and various anilines to generate the thiourea. Further functionalization of the phenylenediamine by amide coupling with various carboxylic acids complete the compound synthesis. Optimization of the synthetic steps including the purification techniques required at each step will be discussed. So far, six members of the thiourea library have been fully synthesized and are being screened for inhibition in a pan-herpes inhibition assay. With optimized synthetic steps and initial inhibitory results, structure-activity relationships will be generated to produce more active inhibitors for further biochemical and *in vivo* studies.

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Abstract 2079

Selected Cytochrome P450 Transcript Expression Analyses in *Candida albicans* in Response to Natural Product, Silymarin

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Jon Mitchell

Candida albicans is an opportunistic pathogenic species of yeast that causes a wide range of infections from oral thrush to systemic candidiasis (Correia et al., 2015). *C. albicans* has an important human health application in that it has developed resistance against commonly prescribed antifungal drugs. Hence, there is a need to explore novel options inhibiting future infections. The field of natural products offers potential treatment alternatives. *Silybum marianum* (milk thistle plant) contains a natural extract called silymarin. Silymarin has demonstrated antifungal effects against *C. albicans* by interacting with the plasma membrane by increasing membrane permeability and inducing oxidative stress (Yun & Lee, 2017). The extract's mode of action, however, remains unclear. This led to the question of whether silymarin might interact directly with certain signal transduction pathways. Cytochrome P450 (CYPs) genes code for enzymes that are highly important in biosynthetic pathways for organismal survival. CYP51, CYP52, and CYP61 were chosen for Quantitative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR) to evaluate levels of transcript expression following treatments with silymarin. CYPs were cloned and overexpressed in *E. coli*. Following separation from recombinant clones, CYPs (+/− silymarin) were analyzed using mass spectrometry to identify potential binding interactions. These interaction analyses may lead to valuable information toward understanding the role of CYPs and the inhibitory mechanism of silymarin against *C. albicans* infections and hopefully lead to expanded drug discovery through natural product compounds.

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Abstract 2114

Exploring derivatives of FDA-Approved Drug, Candesartan, as Potential Inhibitors of PRL3

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Julia Starczewski, Tristan Whalen, Kate Weafer, Mark Vincent dela Cerna

Cancer remains the second most common cause of death in the United States, accounting for more than 600 000 deaths in 2022, with potentially 1.9 million new cases. While there have been significant advancements in cancer treatments, in general, certain types still have high mortality rates and others lack targeted therapies. Metastasis also contributes significantly to cancer mortality. There is, therefore, a critical need to identify novel targets and drugs that can be used as molecular targeted therapies. Aberrant cellular phosphorylation is associated with diseases such as cancers and is a result of dysregulated activities of kinases and phosphatases. As such, these enzyme families present as significant molecular targets. The activity of Phosphatase of Regenerating Liver 3, PRL3 or PTP4A3, has been linked to several oncogenic and metastatic pathways and has emerged as a clinically relevant target for several cancers including breast, ovarian, and colorectal cancers, as well as leukemias. Interest in targeting PRL3 has yielded several experimental inhibitors but none have yet progressed to clinical trials. One approach that has been used to target PRL3 is screening of FDA-approved drugs for possible repositioning. Recently, a screening of a panel of FDA-approved drugs identified candesartan as a potential PRL3 inhibitor. Candesartan is an angiotensin receptor blocker currently used to treat high blood pressure and heart failure. The hydrophobicity of candesartan has prevented detailed characterization of its interactions with PRL3 which is necessary to understand the mechanism of inhibition and to optimize the interactions. Here, molecular docking simulations were used to screen molecules that are structurally similar to candesartan targeting two binding pockets in PRL3. Several candesartan-related molecules were identified that potentially bind to pockets adjacent to the active site, WPD and P, loops. Preliminary assays on one of these molecules, O-desethyl candesartan, revealed that the inhibitory activity against PRL3 is still present. Future studies will characterize the mechanism of inhibition of O-desethyl candesartan and also characterize its interaction with PRL3 as well as assess the ability of other similar molecules to inhibit PRL3 and other members of the PRL family. Overall the ultimate goal of this project is to explore the possibility of using candesartan and its derivatives as a scaffold to develop small molecules that inhibit PRL3, a significant target in cancer metastasis.

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Abstract 2132

Modification of a gene knock out cell line designed for improved analysis of DegQ, a bacterial analog to pregnancy related serine protease (HtrA3)

Natalie Mikita, *Missouri Western State University*

Jennifer Dorris

The High Temperature Requirement A (HtrA) family of enzymes are a diverse group of serine proteases, present in both prokarya and eukarya, and are generally responsible for the degradation of misfolded proteins. The dysregulation of HtrA3 in human somatic cells gives rise to various forms of cancer and preeclampsia. DegQ is a bacterial analog to HtrA3 found in the periplasm of *E. coli* and has the properties of an HtrA protease: it is a serine endopeptidase that works to protect the cell during high temperature by degrading specific damaged proteins. Currently, the interactions that dictate which specific substrates DegQ degrades are unknown. Insights into this interaction could lay the foundation for studying the interactions of the human HtrA3 protease. Previously, the DegQ was cloned into an expression vector (kanamycin resistant pET-24b-DegQ) and transformed into a BL21(DE3) bacterial cell for enzyme production and purification. Initial characterization experiments revealed a self-cleavage pattern that has been identified in a DegQ homolog, DegP, present in the bacterial genome. DegQ and DegP are similar in size and property, and therefore typical purification techniques are incapable of separating them. To create a cell void of DegP and thus purify DegQ without contamination, ΔDegP::kanR *E. coli* from the keio collection were transformed with pBAD-flp to remove the kanamycin resistance gene. Kanamycin sensitive cells were chosen for transformation with pET-24b-DegQ, and kanamycin resistant cells from the transformation were chosen for DegQ purification. Ultimately, analysis of each step was deemed successful, however after growth and purification, DegQ expression was minimal.

We would like to thank the Missouri Western State University departments of Chemistry and biology.

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Abstract 2150

Developing a COVID-19 Yeast Oral Vaccine for Low Income Countries

Christopher Bresnahan, *Providence College*

Nicanor Austriaco, Taylor Brysgel, Noah Kozub

The novel COVID-19 vaccines have been instrumental at transforming the pandemic into an endemic disease. However, many contemporary vaccines, especially the landmark mRNA vaccines, require cold storage that makes them difficult for low income and developing countries to keep and distribute, and no shelf stable, low-cost alternative currently exists. In response to this need, we are developing a novel COVID-19 vaccine delivery system using the probiotic yeast Saccharomyces boulardii. We engineered an integrating construct to express the receptor binding domain (RBD) of the SARS-CoV-2 spike protein tagged with the yeast pheromone secretion signal and with the Claudin-4 targeting sequence of the Clostridium perfringens enterotoxin. Preliminary data from two animal trials suggest that our candidate yeast oral COVID-19 vaccine can trigger a robust humoral immune response in mice. Experiments are underway to assess its effect on the murine T-cell response.

Our laboratory is supported in part by a research grant from the PCHRD-DOST of the Republic of the Philippines.

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Abstract 2158

Development of a spin-label model system for investigation of protein oligomerization states

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Timothy Reichart

The transmembrane domains of viral proteins are highly conserved and crucial to normal viral function. Oligomeric transmembrane domains present novel opportunities for drug development, as their disruption can prevent the assembly of the virus. The Reichart lab is particularly interested in developing retro-inverso peptide inhibitors. Retro-inverso peptides are peptides using D-amino acids mirroring a region of target protein, which allows the peptide to inhibit viral assembly, but they are also significantly less likely to be catabolized by natural metabolic or immunologic processes. The efficacy of these inhibitors is governed largely by the extent to which they mirror the target protein, making highly conserved regions, such as transmembrane domains, ideal target regions for these inhibitors. The primary technique in the literature for the investigation of oligomerization states uses fluorescence spectroscopy. We are now working on developing a novel alternative system to evaluate protein oligomerization using spin-labeled peptides that are directly incorporated into the peptide sequence. Direct incorporation of the spin-label into the peptide sequence is a more powerful technique than the standard procedures used in the literature. In particular, the ability to incorporate spin labels in various positions within the protein can give novel insights into the relative depth of the protein within a membrane, which is very difficult to study using other techniques and not possible using the fluorescence technique. The transmembrane domains of proteins with known and well-characterized monomer and trimer standard oligomerization states were synthesized using an Fmoc Solid-Phase Peptide Synthesis (SPPS) procedure incorporating an Fmoc-2,2,6,6-tetramethyl-N-oxyl-4-amino-4-carboxylic acid, (Fmoc-TOAC) instead of an alanine. Direct incorporation of stable N-oxide spin labels, which can be contrasted to labeling cysteine residues after the protein synthesis, has been used for the investigation of the secondary structure of proteins for decades, but the application of this spin labeling technique to study the oligomerization states of transmembrane domains of proteins is an understudied application. The products of SPPS

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were analyzed using a Liquid Chromatography Mass Spectroscopy instrument and purified using High Performance Liquid Chromatography. The spin-label was then deprotected and evaluated using Electron Spin Resonance (ESR) Spectroscopy. There are two primary future directions following this research project: first, the generation of viral proteins with spin labels incorporated in different positions to determine the relative depth of each position within the membrane; second, the incorporation of spin labels into SARS-CoV-2 proteins to develop a model for *in vitro* evaluation of retro-inverso peptide assembly inhibitors.

-Hampden-Sydney College Office of Undergraduate Research.

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Abstract 2180

Using Synthetic Biology methods to construct a functional estrogen biosensor based on split Nanoluciferase activity

Debora Edouard, *Simmons University*

Grace Solomon, Jennifer Roecklein-Canfield

The presence of estrogenic compounds (endocrine-disruptors, EDCs) in the water supply raises concerns about human and aquatic health. Current methods for detecting estrogen contamination require expensive, time-consuming techniques such as liquid chromatography-mass spectrometry and high-performance liquid chromatography. Previously reported estrogen biosensors required multiple cloning and transformation steps for successful detection in bacteria. Synthetic biology allows for the construction of genetic devises composed of DNA sequences modified to be interchangeable and provide novel functions. New tools and devices are constantly needed to enhance the already extensive list of novel genetic parts. Our approach to the design of an estrogen responsive element uses methodology developed in the Wells lab (Elledge et al, 2021) to detect SARS-CoV-2 antibodies. This methodology takes advantage of the split Nanoluciferase (spLUC) protein divided into two functional domains (designated SmBit and LgBit). Based on rational engineering design we express dimerization dependent LgBit and SmBit fused to the Estrogen Receptor alpha protein (ERalpha) in bacteria cells. These two monomeric proteins will dimerize in the presence of estrogen, reconstitute the split luciferase enzyme and reestablish enzyme activity. Cells can be lysed, and luminescence detected to quantify estrogen present in the sample. We present here the construction strategy and proof of concept data demonstrating the efficiency of this dual-functional biosensor and its effectiveness for detection of estrogenic compounds in contaminated water.

NSF-REU-1852150, REU Site: A multisite REU in Synthetic Biology, 2019.

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Abstract 2209

A Molecular Dynamics Study of Product Release from the Yeast Cytosine Deaminase Enzyme

Kayla Croney, *Western Washington University*

Jay McCarty

Characterizing binding affinities and the complete set of ligand binding-unbinding pathways is a key step in the design of novel drugs and engineered enzymes. Atomistic molecular dynamics (MD) simulations can provide important information about the role of protein conformational dynamics on receptor-ligand interactions. In this work we perform extensive MD simulations of the yeast cytosine deaminase (yCD) enzyme. The yCD enzyme is of interest in cancer gene therapy because it can convert the prodrug 5-fluorocytosine (5-FC) into an active anticancer drug 5-fluorouracil (5-FU). However, the release of the product is the rate-limiting step, limiting the effectiveness of the prodrug-enzyme strategy. We use the recently developed volume-based metadynamics approach to sample accessible binding-unbinding pathways. Using this enhanced sampling approach, we characterize several unbinding events and present the free energy surface for the binding/unbinding of 5-FU and uracil from yCD. We identify a possible metastable intermediate for 5-FU, which could potentially be responsible for the slow product release. In addition, we calculate the binding free energies for both enzyme products.

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Abstract 2232

Heterologous Production of the D-Cycloserine Intermediate O-acetyl-L-serine in Human Lung Cancer Cells

Laurel Robbins, *Lake Forest College*

Ariane Balaram, Stefanie Dejneka, Matthew McMahon, Zarina Najibi, Peter Pawlowicz, William Conrad

According to the World Health Organization, Tuberculosis (TB) is the second leading cause of death by a single infectious disease behind COVID-19. Despite a century of effort, the current TB vaccine does not effectively prevent pulmonary TB, promote herd immunity, or prevent transmission. Therefore, we seek to develop a genetic prophylaxis for TB. We have determined D-cycloserine to be the optimal target for this approach due to its relatively short six-enzyme biosynthetic pathway. D-CS is a second-line antibiotic for TB that inhibits bacterial cell wall synthesis. The first committed step towards D-CS synthesis is catalyzed by the L-serine-O-acetyltransferase (DcsE) which converts L-serine and acetyl-CoA to O-acetyl-L-serine (L-OAS). To test if the D-CS pathway could be an effective prophylaxis for TB in human cells, we endeavored to express DcsE in human cells and test its functionality. We overexpressed DcsE tagged with FLAG and GFP in A549 lung cancer cells as determined using fluorescence microscopy. We observed that purified DcsE catalyzed the synthesis of L-OAS as observed by HPLC-MS. Therefore, DcsE synthesized in human cells is a functional enzyme capable of converting L-serine and acetyl-CoA to L-OAS demonstrating the first step towards D-CS production in human cells.

103311, https://doi.org/10.1016/j.jbc.2023.103311

Abstract 2235

Cytotoxic and anti-migratory effects of a benzylamine epoxide product in triple-negative and early-stage breast cancer cells

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Jeff Hansen, Sarah Mordan-Mccombs

Introduction: Breast cancer is currently one of the most diagnosed and deadly cancers in women around the world with 2.3 million women diagnosed and 685 000 deaths. Early-stage treatments such as selective estrogen receptor modulators and antibody-based therapies have proven effective to increase survival and prolong the time to relapse, but there are few options for late-stage triple-negative breast cancer (TNBC). Thus, current research is focused on chemopreventive and chemotherapeutic agents for late-stage disease. Dr. Jeff Hansen's lab at DePauw University has found evidence that their epoxide-derived β-amino alcohol compounds are potential targets for cancer treatments due to their cytotoxic effects on brine shrimp and the HL-60 leukemia cell line. The benzylamine and epoxide product synthesized in this library and tested here has an LC50 value of 8.28 uM in the brine shrimp lethality assay, which indicates that the compound is potentially cytotoxic at low doses against cancer cells.

Methods: In order to investigate the effects of the epoxide-derived products on breast cancer, cell lines SUM159 (TNBC) and MCF7 (early-stage breast cancer) were treated with potential cytotoxic compounds. Crystal violet assays were used to determine the effects on cell viability, wound healing assays to observe effects on migration, and flow cytometry to verify apoptosis of the cells. *Saccharomyces cerevisiae* knock-outs of non-essential genes in the oxidative stress response pathway were grown in the presence of the epoxide products and hydrogen peroxide to find the possible mechanisms of cytotoxicity.

Results: In both the SUM159 and MCF7 cell lines, the cell viability was decreased in the treated wells in comparison to the control wells across concentrations. Effects of the compound on HEK293T cells are also assessed. The *Saccharomyces cerevisiae* had decreased cell growth when treated with the benzylamine product and hydrogen peroxide in comparison to the control in Δyap1, Δtsa, Δaif1, and Δzwf1 deletion mutants.

Discussion: The results indicate that the benzylamine product is a potential antitumor drug because of its cytotoxic effects against the SUM159 and MCF7 breast cancer cell lines. Further, the drug appears to have anti-migration effects, as determined by a wound healing assay, indicating potential inhibition of metastasis. The *Saccharomyces cerevisiae* experiments indicate that oxidative stress induction may contribute to the epoxide product's mechanism of cytotoxicity. In addition, flow cytometry of treated cells shows the ability of the epoxide products to induce apoptosis or necrosis in the breast cancer cell lines.

This project has been funded by a Science Research Fellows grant from DePauw University to RH and New Faculty Start-Up funding to SMM.

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Abstract 2267

Identification of Recognition Sites in the Liver Drug Transporter OATP1B3

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Taiwo Awe, Lanie Gardner, Kehinde Awe

The transport proteins Organic Anion Transporting Polypeptide 1B1 and 1B3 (OATP1B1 and OATP1B3) are located at the basolateral membrane of human hepatocytes and mediate the uptake of drugs like statins and antibiotics into these cells. These proteins are made up of 12 transmembrane domains (TMs) and share numerous substrates. However, each of the proteins has some unique substrates. We specifically focused on Indocyanine Green (ICG), a substrate that is unique to OATP1B3. ICG is used in medical diagnostics for intraoperative liver imaging to determine hepatic function. Both OATP1B1 and OATP1B3 transport substances from the blood into the liver, and regulate how medication interacts with hepatocytes. Many studies have determined the uptake of drugs throughout the body and identified OATP1B3's distinctive role. Our goal was to understand the function, structure, and the importance of the Organic Anion Transporting Polypeptide (OATP1B3) in the human body. Specifically, we wanted to test the importance of individual TM domains for the uptake of ICG. To image this protein and understand the structure, the website AlphaFold was used. To test the importance of the individual TMs, we transfected HEK293 cells with plasmids encoding full-length OATP1B1 or OATP1B3 and chimeric proteins, where individual TMs were swapped. Forty-eight hours after transfection we measured the uptake of 1 uM ICG for 1 minute and corrected the values for total protein. Presented here is a physical model illustrating the twelve transmembrane domains present in OATP1B3. Uptake of ICG was similar to OATP1B3 in most chimeric proteins except for TM2, TM9 and TM10 which showed reduced uptake. ICG uptake by OATP1B1 was essentially absent. Research was conducted on a potential central pore located around Transmembrane Domain 2 and Transmembrane Domain 10. Each of the transmembrane domains play a crucial role in the uptake of ICG, with transmembrane domains 2, 5, 9, and 10 showing an increase in uptake when transfected with individual TMs of OATP1B3.

This MAPS Team project is supported by the Center for Biomolecular Modeling and the Medical Professions Academy at Olathe North High School under the mentorship of Dr. Bruno Hagenbuch, University of Kansas Medical Center, Kansas City, KS.

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Abstract 2272

Structural and biochemical characterization of flavone 3'-O-methyltransferase from *Oryza sativa* (rice)

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Soon Goo Lee, Sophie Spaulding

S-adenosyl methionine (SAM)-dependent methyltransferases are a class of enzymes involved in the metabolism of xenobiotic and endogenous compounds, gene transcription, and methylation of biomolecules. Specifically, flavone 3'-O-methyltransferase (OsMT) plays a vital role in the flavone biosynthesis pathway in *Oryza sativa* (rice). The three-dimensional (3D) structure of OsMT has provided a molecular basis for the characterization of its enzymatic function and potential applications. The biochemical activities of OsMT and the roles of active site residues were investigated using a combination of molecular biology and biochemical techniques. Multiple point mutations were generated via QuikChange PCR mutagenesis, and the resulting proteins were expressed as His-tagged fusion proteins and purified by nickel-affinity chromatography to examine the effect of changes in the substrate and cofactor binding sites. Kinetic analysis was used to analyze the effect of mutations on activity and substrate specificity. Results provide insights into the contribution of active site residues to OsMT function.

We would like to acknowledge and thank the UNC-Wilmington Center for the Support of Undergraduate Research and Fellowships (CSURF) for supporting funds, as well as Argonne National Laboratory for the use of their resources and technology.

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ASBMB

Abstract 2282

Building a molecular toolkit for bioengineering of the lignin biosynthetic pathway in *Chlamydomonas reinhardtii*

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As atmospheric carbon dioxide concentrations increase, it is critical to develop new tools for long-term sequestration of atmospheric carbon. Our goal is to modify the genome of the single-celled algae *Chlamydomonas reinhardtii* to facilitate the biosynthesis of extracellular lignin polymer. We plan to insert genes using Cas9-directed double-stranded DNA cleavage and homologous directed recombination. Previous literature has demonstrated that the genomic DNA of *C. reinhardtii* can be cleaved by Cas9/sgRNA RNPs which are introduced into the cells by electroporation. As a first step in our pathway engineering, we aim to increase flux through the phenylpropenoid pathway by introducing a gene encoding for phenylalanine tyrosine ammonia lyase (PTAL) from *Chryseobacterium luteum*, which performs the first committed step in the biosynthesis of lignin precursors. We modified the *C. luteum* PTAL coding sequence to optimize codons and to include introns and 5' and 3' UTRs optimized for high level gene expression in *C. reinhardtii*. In parallel, we designed and tested gRNAs targeting the NIT1 gene on chromosome 9 to provide a new locus for gene insertion. In order to identify the impact of our genome modifications on metabolic flux, we developed a gas chromatography/mass spectrometry (GC-MS) assay to derivatize and identify key monolignol precursors. Once we achieve these proof-of-principle milestones, we will be able to apply these tools to test the impact of inserted lignin biosynthesis genes on the concentration of monolignols produced by *C. reinhardtii*, and to identify optimal sites in the genome for high level expression of inserted genes.

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Abstract 2288

Computer-aided design of novel small-molecule inhibitors of hAtg7 E1 enzyme

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Post-translational modifications regulate various biological processes, including recognition and modification of ubiquitin (Ub) and ubiquitin-like proteins (Ubls). In particular, E1 enzymes, such as Atg7, catalyze activation of two Ubls, Atg8 and Atg12. The Ubls are distinctly conjugated to E2 enzymes Atg3 and Atg10, respectively. Atg7 plays a key role in eliciting autophagic responses. Dysfunction of the Atg7 pathway is associated with neuropathological disorders, infections, and cancer, hence, making it an important therapeutic target in biology and medicine. Atg7 is structurally and functionally unique from the canonical E1 enzymes. Molecular mechanisms driven by human Atg7 (hAtg7) remain unclear. The X-ray crystal structure of yeast Atg7 has been resolved, representing a homodimer composed of 630 residues, including the N-terminal domain (NTD) and C-terminal domain (CTD) connected by a short linker. In this study, we aim to develop novel inhibitors that target Atg7 as an effective approach for enzyme inactivation through virtual screening and biochemical experiments. We will build a homology model of the hAtg7 using the yeast Atg7 structure (PDB: 3VH2) as a template. We will perform virtual screening of the ChemBridge CORE library against the structural ensemble of hAtg7. LigPrep in Schrodinger will be applied to prepare the ChemBridge compounds and AutoDock flexible docking will be performed on the hAtg7 structural ensemble. We will test ∼30–50 top-ranked compounds carefully chosen from the virtual screening in collaborative biochemical experiments.

This MAPS Team project is supported by the Center for Biomolecular Modeling and the Medical Professions Academy at Olathe North High School under the mentorship of Dr. Yinglong Miao and Shristi Pawnikar, University of Kansas, Lawrence, KS.

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Abstract 2289

Virtual Screening of Mur-E Inhibitors via Conformational Ensemble Generation

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Antibiotic resistance is an ever-growing threat, which necessitates the development of novel antibiotics. Peptidoglycan (PTG) is an ideal target for antibiotic development as it is necessary for the structural integrity of the cell and is unique to bacterial cells. Mur-E, a cytoplasmic ligase, is an integral component of PTG synthesis and shares many conserved structures with PTG ligases in other bacterial species. Thus, inhibitors of Mur-E are likely to be effective broad-spectrum antibiotics. Mur-E's role in PTG synthesis is to add meso-A2pm or L-lysine to a nucleotide precursor of PTG, UMAG; this reaction is facilitated by ATP hydrolysis. This project aims to use ClustENMD, a coarse-grained elastic network model-based methodology, to generate a conformational ensemble and dock a series of potential inhibitors to analyze the change in binding due to conformational changes in the protein. This approach allows for cryptic pockets to be identified that may bind well to some ligands, potentially allowing for the development of allosteric inhibitors of the protein. Using a combined approach of conformational sampling and traditional docking using AutoDock Vina, we aim to gain greater insight into how the dynamics of Mur-E affects inhibitor development, allowing progress to be made in the design of novel antibiotics.

Milwaukee School of Engineering and the Chemistry & Physics Department provide any needed funding for our research.

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Abstract 2331

Developing Alginate-Based Hydrogels for the Delivery of Magnetic Hyperthermia Nanoparticles

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Yiwei Wang, Marcos Garcia, Sibani Biswal, Ahmed El-Gendy

Magnetic hyperthermia therapy is a cancer treatment that uses magnetic nanoparticles (MNPs) to induce localized heating to suppress tumors. This is achieved by Neel relaxation - a coupling between magnetic field & moment - which causes heat dissipation. Thus when a high-frequency external magnetic field is applied to MNPs, local tumor suppression occurs. Although research in the field has made many strides over the past two decades, many challenges remain in developing a suitable comprehensive clinical treatment plan, including developing durable delivery methods, guiding MNPs to tumor sites, appropriate application of external current, and the extraction of MNPs after therapy. Our project focuses on developing a food-grade alginate-based hydrogel encapsulation-delivery system for iron oxide MNP delivery. This was achieved by simple methodologies, utilizing a classical precipitation reaction in an aqueous environment to successfully incorporate MNPs in an alginate hydrogel. The proposed hydrogel delivery mechanism provides multiple advantages; primarily in biodegradability and health compatibility, but also including ease and affordability of manufacturing, and a high potential for coupling with both chemotherapy-specific as well as general drug delivery.

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Abstract 2334

Non-Saccharide Glycosaminoglycan Mimetics Selectively Target Certain Receptor Tyrosine Kinases to Induce Anti-Cancer Stem Cell Activity

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Shravan Morla, Ravi Kumar Ongolu, Nehru Viji Sankaranarayanan, Nirmita Patel, Rio Boothello, Bhaumik Patel, Umesh Desai

Glycosaminoglycans (GAGs) are a unique class of natural polysaccharides that exhibit a wide range of activities that contribute to both physiologic and pathologic growth. Unfortunately, the roles of these molecules are poorly understood due to their polydispersity, microheterogeneity and synthetic challenges. Our lab has advanced the concept of fully synthetic, non-saccharide glycosaminoglycan mimetics (NSGMs) as chemical probes of functions of natural GAGs. Recently, we identified that HS06, a heparan sulfate hexasaccharide, preferentially inhibits the growth of colorectal cancer stem cells (CSCs) cultured as 3-D spheroids. Computational studies suggested that G2.2, a chemically synthesized NSGM, could structurally and dynamically mimic HS06. Further, G2.2 displayed selective and potent activity against CSCs, which provide additional evidence that structural mimicry of HS06 transforms into functional mimicry too. To advance the CSC targeting capability of G2.2, three lipid-modified analogs (LMAs) were synthesized. All three LMAs showed enhanced potency in targeting CSCs compared to bulk tumor cells with nM spheroid inhibition reached for one of the analogs. To identify the putative cellular target(s) of G2.2 and its LMAs, a phosphoreceptor tyrosine kinase array consisting of 49 possible targets was used. G2.2 was found to reduce the activation of insulin-like growth factor-1 receptor (IGF-1R) preferentially over a related NSGM control that does not inhibit CSCs. Biophysical studies showed that for proteins belonging to the IGF-1R system, G2.2 shows over a 3.6-fold binding affinity preference for IGF-1R in comparison to its growth factor ligand IGF-1[BP1]. Interestingly, the LMAs also preferentially engaged IGF-1R over IGF-1, while simultaneously displaying enhanced affinity for IGF-1R compared to parent molecule (G2.2). Furthermore, out of a panel of more than a dozen growth factor receptors relevant for stemness and GI tumors, VEGFR1 and TGFβR2 were found to be possible targets of all four NSGMs. Biophysical and computational studies, especially with a group of mutant proteins, pinpoint plausible site of binding of the NSGMs. In combination, our detailed results show for the first time that NSGMs show selective pleiotropicity for receptor tyrosine kinases. Overall, NSGMs represent unique anti-cancer agents that selectively inhibit a tumor-initiating subpopulation of cancer cells, thereby possessing major potential to provide long-term remission for cancer patients, especially with gastrointestinal cancers. [BP1]Is this correct? Just seems different than HS06.

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Abstract 2337

Development of a rapid screening platform for the bacterial cell wall recycling enzyme AmgK

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Ashlyn Hillman, Kimberly Wodzanowski, Ha Le, Ashu Sharma, Catherine Grimes

Peptidoglycan (PG) recycling pathways have been broadly expressed throughout different bacterial species to serve as a means of conserving resources and limiting energy expenditure from the taxing process of PG biosynthesis. For example, N-acetylmuramate/N-acetylglucosamine kinase, AmgK, and N-acetylmuramate alpha-1-phosphate uridylyltransferase, MurU, recycling enzymes found in the bacteria *Pseudomonas Putida*, catalyze the production of UDP-N-acetyl muramic acid (NAM) from NAM. Our lab developed a metabolic labeling strategy that implements these recycling enzymes into *Escherichia coli*, which do not naturally express such recycling machinery, as a technique to incorporate modified NAM derivatives into PG biosynthesis and subsequently bacterial cell walls. To evaluate if NAM derivatives are suitable for metabolic labelling experiments PpAmgK was purified and used in an NADH coupled ATPase assay. Here we have increased throughput by converting the assay to a semi-automated plate-based platform. This new approach substantial decreases experimental preparation and runtime (~20 mins), allows for obtaining kinetic data for up to 3 NAM derivatives in triplicate in a single run, and proves to be highly reproducible. Using this platform, we continue to explore the AmgK that have been identified in different bacterial species, such as *Tannerella forsythia*, an oral pathogen. Phenotypically we observed that the incorporation of NAM derivatives is more efficient when using Tannerella AmgK and MurU; we will utilize this new high-throughput screening platform to kinetically analyze the enzymes. We set to determine if this trend relates to its kinetic and substrate specificity profiles. We foresee this method will prove invaluable for rapid determination of new NAM derivatives as well as an ideal platform for screening inhibitors of AmgK.

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Abstract 2348

Investigating the role of furin in the intoxication pathway of recombinant immunotoxins, based on *Pseudomonas exotoxin* A

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John Weldon

The serine protease furin cleaves and activates both endogenous proteins and proteins from pathogens, including the bacterial toxin *Pseudomonas exotoxin* A (PE). We are investigating the role of furin in the intracellular trafficking of PE-based recombinant immunotoxins (RITs), protein immunoconjugates designed for targeted cell killing. While furin is recognized for its proteolytic activity, it has also been shown to act as non-proteolytic chaperone. Based on studies of furin trafficking and its cleavage site preferences, as well as current understanding of the intoxication pathway of PE, we hypothesize that furin can act as a chaperone for PE and PE-based RITs. I am using a PE-based RIT targeted to the transferrin receptor to address our hypothesis in two aims. First, I am investigating the interaction between furin and RITs using *in vitro* co-immunoprecipitation. Preliminary results indicate a stable interaction between furin and RIT. Second, I plan to observe the localization of furin and RITs during intoxication using subcellular fractionation and confocal microscopy. Information from this investigation will allow us to improve the efficacy of RITs as therapeutic agents against cancer.

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Abstract 2360

Anaebena Sensory Rhodopsin Membrane Protein and ApoA1 Detection via Western Blot Analysis

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Leila Amrani, Jenny Cappuccio

Anabaena sensory rhodopsin (ASR), originally isolated in a cyanobacterium currently known as Nostoc, is a photoreceptor within the G protein coupled receptor (GPCR) family. Since GPCRs are embedded into the lipid membrane and contain both hydrophilic and hydrophobic regions, it is difficult to study their native activity. Nanodiscs serve to create an environment where membrane proteins can be stabilized in a natural amphipathic environment. In the present study, 6x-His ASR was isolated, purified by Ni-NTA affinity chromatography, and assembled into a lipid rich nanodisc with the ApoA1 scaffold protein and DMPC lipid. The purified ASR and ASR nanodisc was studied by spectral, SDS-PAGE, Native-PAGE, and chemiluminescent western blotting analysis. Here we report that the ApoA1 scaffold protein and the ASR membrane protein were successfully detected in the ASR nanodisc by western blot analysis. By SDS-PAGE, we found that our buffer exchanged ASR nanodisc was composed of ApoA1 scaffold protein (22.81 kDa) and a less prominent band for ASR (18.36 kDa). A distinctive and sharp band was observed for buffer exchanged ASR (18.36 kDa), suggesting presence of the protein. By the chemiluminescent SDS-PAGE western blot with the primary Anti-ApoA1 and secondary HRP antibodies, we detected strong chemiluminescence in the ASR nanodisc, the ApoA1 control, and the ASR nanodisc wash aliquot. By chemiluminescent SDS-PAGE & Native-PAGE western blot investigation with the anti-6x His primary and secondary antibody, we detected ASR in the ASR nanodisc and the buffer exchanged ASR aliquot. These results suggest that Apolipoprotein A1 and ASR are present in the nanodisc. Additionally, the primary and secondary antibodies were able to bind as expected. Next steps would include replicating these findings and improving the Native-PAGE western blot with the anti-ApoA1 primary antibody.

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ASBMB

Abstract 2410

Towards the X-ray Crystal Structure of an Iridium Complex bound to a DNA G-Quadruplex

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Kali Cook, Jean Jakoncic, Carly Reed, Michael Gleghorn

The immediate goal of this research is to find the binding conditions for the iridium-containing ligand (RHH111319) given to us by our collaborator that permits interaction with guanine-rich G-quadruplex (G4) structures in telomeric or oncogenic regions of DNA sequences, such as in the c-MYC promoter. Our goal is to produce a crystal structure of this ligand-DNA complex. Iridium is a low-toxicity transition metal, and when it is bound to certain organic molecules, the resulting complex can selectively bind to G4 DNA, which is a target in cancer cells in humans. This can lead to the discovery of the role G4 DNA plays in cancer development. Cyclometalated iridium (III) compounds, like ours, are currently used in cancer research for biological imaging. This is because of their high quantum yield, long periods of luminescence, and antitumor activity. For co-crystallization of the ligand and G4 DNA, both molecules must ultimately be dissolved in the same solution. This has proven to be very difficult because the preferred solvent for the iridium-containing compound often destroys the G4 crystal or causes the ligand to precipitate. Rather than co-crystallizing, we first attempted crystallizing the G-quadruplex using published conditions, followed by soaking these crystals in a solution containing ligand. Soaking experiments were conducted and analyzed via X-ray diffraction at Brookhaven National Laboratory using their National Synchrotron Light Source II. After collecting many datasets as high as 1.66Å resolution, and with different soaking conditions, we have yet to find extra electron density for the ligand. The data does however show variations of a known G4 structure, with differing unit cells, space groups, and higher resolutions, suggesting potential improvements and/or corrections of the model. The crystal structure has end-to-end interactions of G4 helices and we think this might preclude ligand binding. Consequently, the ligand and G4 must first bind together in solution, and co-crystallize once bound. Work is now being conducted to determine these co-crystallization conditions in order to perform a new round of X-ray diffraction experiments towards determining this crystal structure. From such a structure, we will better understand the precise G4 interactions of the iridium-complex targeting molecule.

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Abstract 2414

Aurone-derived 1,2,3-triazoles as anti-inflammatory agents *in vitro*

Daniel Bryant, *Middle Tennessee State University*

Arjun Kafle, Sydney Ferguson, Scott Handy, Anthony Farone

Chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease are characterized by a dysregulation of the immune system leading to increased levels of proinflammatory cytokines, chemokines, and other inflammatory markers such as reactive nitrogen species. 2-Benzylidene-1-benzofuran-3(2H)-ones (aurones) are a type of naturally occurring flavonoid responsible for the yellow pigmentation in certain flowering plants and have been shown to have anti-inflammatory, antibacterial, and anti-cancer properties. In 2020, in an effort to add an azido group to an aurone, Kafle *et al*. serendipitously synthesized a new derivative of aurone which contains a 1,2,3-triazole in its backbone. Since 1,2,3-triazole containing compounds have previously been shown to regulate inflammation, it was hypothesized that these new aurone-derived 1,2,3-triazoles (ATs) could potentially function as anti-inflammatory agents. To test this, various ATs were screened for their anti-inflammatory and cytotoxic activity using lipopolysaccharide (LPS) stimulated RAW 264.7 murine macrophage-like cells. Two ATs (AT5 and AT111) which had anti-inflammatory activity and caused minimal reduction in cell viability were further examined using ELISAs, western blotting, fluorescent microscopy, and RNA sequencing. Both AT5 and AT111 decreased levels of secreted nitrite in a dose dependent manner in RAW264.7 cells cotreated with ATs and LPS for 24 h and similarly in those pretreated for 4 h prior to addition of LPS. At lower concentrations, both ATs caused an increase in nitrite compared to the positive control. Despite the apparent reduction of nitrite levels, only AT111 was shown to decrease levels of inducible nitric oxide synthase (iNOS) on a protein level. However, neither AT attenuated levels of cyclooxygenase 2 (COX2). Both ATs were also able to decrease levels of proinflammatory cytokines. Differential gene expression analysis showed that AT111, but not AT5, caused a downregulation of multiple proinflammatory response genes including many interleukins such as *Il1α/β*, *Il6*, and *Il15*, as well as *Nos2* in LPS stimulated RAW 264.7 cells. AT111 also downregulated genes associated with an inflammatory response in LPS treated differentiated human U937 cells potentially indicating transcriptional regulation of the LPS induced proinflammatory response. AT111 was also shown to increase expression of genes associated with nucleosome assembly/organization and chromatin remodeling in both human and murine cell lines. Analysis of RAW 264.7 *Nos2* reporter cells, which utilize a region of the *Nos2* promoter with NF-κB and STAT1 enhancers to control expression of mCherry, showed no difference in relative fluorescence units between the positive control and AT111 and AT5 treatments, potentially implying

that the ATs regulate inflammation possibly in an NF-κB and STAT1 independent manner. This work is the first to characterize the anti-inflammatory potential of these novel ATs through downregulation of inflammatory response genes. The regulation of some inflammatory mediators but not others induced by LPS indicates that these ATs may be influencing the inflammatory response downstream potentially through an NF-κB independent manner, possibly through modulation of genes related to chromatin remodeling and nucleosome organization, though further research is required.

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Abstract 2438

The immortal jellyfish: a (POT1)ential victory over death

James Greig, *Ashbury College*

Zoe Fedoruk, Mackenzie Delilah Hao, Alicia Huang, Chloe Li, Ethan Peacock, Angel Shi, Jaya Thacker, Benjamin Woo, Tristen Zhang

The "immortal jellyfish" (*Turritopsis dohrnii*) is one of a few cnidarian species that can avoid mortality by reverting its life cycle from a medusa – its adult stage, to a polyp – its juvenile stage – through a process called transdifferentiation using its stem cell reserves. In normal somatic cells, telomeres, which are the ends of chromosomes that protect DNA, shorten with age. When telomeres reach a critical length, the cell will undergo apoptosis, senescence, or tumorigenesis. Meanwhile, in germline and stem cells, the enzyme telomerase maintains telomere length by adding TTAGGG repeats to the 3' telomeric single-stranded DNA overhang using its complementary template. Recruitment of telomerase by the protein complex shelterin allows for the binding of telomerase to telomeres, enabling telomeric transcription and elongation. Shelterin helps protect telomeres by regulating telomere length. It is composed of six protein subunits including Telomere Protection Protein 1 (TPP1) which connects to telomerase, and Protection of Telomeres 1 (POT1). POT1 binds to single-stranded 5'-TTAGGG-3' telomeric tandem repeats in eukaryotic species, inhibiting telomerase binding and disabling telomere elongation. Mutations of POT1 in *T. dohrnii* at positions H266L, G272N, and S322L weaken POT1's ability to bind to telomeres and increases telomerase activity, potentially allowing the cell to live indefinitely. A better understanding of POT1's role in shelterin, and the mutations that differentiate POT1 in *T. dohrnii* and humans may give insight into aging, regenerative techniques, and disease prevention. For this project, the Ashbury College Center for BioMolecular Modeling A Protein Story (MAPS) team used 3D modeling and printing technology to model the binding of telomerase to the TPP1-POT1 heterodimer, and the unique mutations of POT1 in *T. dohrnii* compared to humans.

For this project, the Ashbury College Center for BioMolecular Modeling A Protein Story (MAPS) team used 3D modeling and printing technology to model the binding of telomerase to the TPP1-POT1 heterodimer, and the unique mutations of POT1 in *T. dohrnii* compared to humans.

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ASBMB

Abstract 2451

(MOR) than one binding site, leaves Naloxon

James Greig, *Ashbury College*

Elfin Duru Akteke, Kevin Bai, Grace Cooper, Laura Delage, Natalie Feberova, Sara Monteith Adams, Adam Mulugeta, Morgan Powter, Lauren Rasalingam, Will Trainor, Ariam Biniam, Lorena Velásquez

Opioid abuse in North America is classified as an epidemic, resulting in over 760 000 opioid-related deaths since 1999. Although the number of opioid prescriptions have decreased in Canada from 2013 to 2018, it is still a highly prescribed class of medication in the U.S., notable for their efficacy in pain-modulation via the inhibition of pain signals. Due to their characteristic ability to induce euphoria alongside their desired pain-management effects, the abuse and dependence on opioids is a consequence of their frequent application. Opioids are a broad group of analgesic drugs that target the body's 3 main opioid receptors: μ (mu), κ (kappa) and δ (delta). Integral to the function of exogenous opioid drugs is their affinity for interaction with the μ-opioid receptors (MOR), a type of G protein-coupled receptor (GPCR) abundant in the central and peripheral nervous system to which they bind agonistically to elicit analgesia. Naloxone is an opiate antagonist, with a half life of approximately 2 hours, that is used as a temporary antidote to reduce the effects of opioid overdoses and counteract the life-threatening depression of the respiratory system and central nervous system (CNS). The drug has a greater affinity for the μOR1 receptor than opioids, and therefore fewer receptors remain open to binding the opioid. Unlike opioids, naloxone does not activate the receptor, and therefore it can bind without having negative effects on the CNS. The Ashbury College Center for BioMolecular Modeling A Protein Story (MAPS) team used 3D modelling and printing technology to model the μ-opioid receptor to which these agonists and antagonists bind to.

The Ashbury College Center for BioMolecular Modeling A Protein Story (MAPS) team used 3D modelling and printing technology to model the μ-opioid receptor to which these agonists and antagonists bind to.

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Abstract 2452

Optimization of a Genetically Encoded Fluorescent Mg2+ Sensor Platform to Illuminate Cellular Mg2+ Distribution and Dynamics

Evan Pratt, *Northern Michigan University*

Wren Konickson, Joey Bogaudo, Hailey Lightle

Mg2+ is one of the most abundant cellular metal ions and governs the structure and function of a wide range of biological molecules, including nucleic acids, cellular metabolites and proteins. Mg2+ is integral to supporting nerve and muscle function, the immune system and bone health, and dysregulation of Mg2+ homeostasis is associated with the development of cardiovascular disease, diabetes and osteoporosis. Despite the well-accepted cellular and physiological role of Mg2+, the behavior of Mg2+ at the single cell level is ill-defined. Genetically encoded fluorescent sensors have been used to illuminate the behavior of metal ions, such as Ca2+ and Zn2+, in cells with remarkable spatiotemporal resolution. However, there is a dearth of fluorescent tools that can be used to interrogate Mg2+ biochemistry in cells. In this study, we engineered three FRET-based Mg2+ sensor platforms that contain distinct Mg2+-binding domains (MBD) inserted between the same two fluorescent proteins: ECFP and cpVenus173. Two of the major challenges impeding the development of a robust fluorescent Mg2+ sensor are Mg2+-selectivity and sensor responsiveness. Since the MBD couples metal ion binding with fluorescence changes, this component of the sensor requires our attention. The MBDs interrogated in this study were derived from different EF hand proteins: Centrin-3 (Cen3), cardiac troponin C (cTnC) and the neuronal Ca2+-binding protein, S-CaBP-1. Each protein contained two EF hands - motifs that are normally associated with Ca2+ binding but can coordinate Mg2+ with moderate affinity. After constructing each fluorescent sensor by molecular cloning, we expressed them in bacteria and purified them using Ni2+-NTA column chromatography. To assess sensor responsiveness, we collected fluorescence emission spectra in the presence of 10 mM Ca2+ or Mg2+. Each of the three Mg2+ sensor platforms demonstrated a subtle response to one or both metal ions. In an attempt to improve coupling between metal binding and fluorescence changes, we truncated the region connecting Cen3 or cTnC with the fluorescent proteins. The truncated version of the Cen3-based sensor, but not the cTnC-based sensor, exhibited a robust response to both metal ions. To evaluate binding specificity, we titrated the truncated Cen3-based sensor with Mg2+ or Ca2+ (1 μM–10 mM) and determined the apparent dissociation constant (Kd') for Ca2+ and Mg2+ binding. We found that the Kd' for Ca2+ and Mg2+ binding were 97.5 ± 15.7 μM and 1.38 ± 0.183 mM, respectively, indicating that the sensor had a greater affinity for Ca2+. To manipulate metal ion binding, we targeted a glutamate residue within one of the EF hands, given its documented importance in

Ca2+ coordination, and mutated this residue to aspartate (E12D) or glutamine (E12Q). The truncated Cen3-based sensor containing E12D showed a 39.5-fold reduction in Ca2+ binding with a modest 3.7-fold decrease in Mg2+ affinity. Strikingly, E12Q reduced Ca2+ affinity by 24.5-fold with no apparent change in Mg2+ binding. Thus, the truncated version of the Cen3-based sensor containing the E12Q mutation is a promising candidate for detection of cellular Mg2+.

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Abstract 2476

Structural basis for condensation domain independent amide bond formation in lincosamide antibiotic biosynthesis

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Condensation (C) domain independent nonribosomal peptide synthetases (NRPSs), as found in the lincomycin/celesticetin and petrobactin pathways, rely on a variety of peptide forming enzymes (PFEs) to generate an amide bond from an amine and a carrier protein bound thioester via a phosphopantetheine prosthetic group. The mechanism of amide bond formation and the interactions with their cognate carrier proteins have not been elucidated. We investigated this by crystallization of CcbD, the PFE from the biosynthesis of celesticetin, a protein with no similarity to any known protein family in complex with its carrier protein. The structure revealed a homodimeric complex with a cysteine essential for turnover of carrier protein thioester, typical electrostatic interactions along helix 2 of the carrier protein, and an unusual tryptophan gating mechanism. This tryptophan forms a cap over the active site in the absence of the carrier protein but flips into a hydrophobic pocket in center of the carrier protein helix bundle upon binding. This interaction is further stabilized by the gem-dimethyl group of the phosphopantetheine prosthetic group. Mutational analysis of this position revealed that this flipping is essential for efficient turnover and likely enforces chain flipping and funneling of the thioester into the active site. A recently published structure of AziU3, an aziridine synthase with low sequence homology, was found to adopt a highly similar fold. AziU3 does not form an amide bond nor interact with a carrier protein, rather forming a heterodimer with AziU2, a beta-sandwich protein. Overall, the structures demonstrate a new fold for amide bond formation in NRPS systems which can serve diverse functions and may aide future engineering efforts of these unusual pathways.

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Abstract 2478

Chemical probes for accelerating the analysis of drug targets in *Mycobacterium tuberculosis*

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M. tuberculosis (Mtb) is the causative agent of tuberculosis (TB), the most deadly infectious disease in human history. The 10 million new TB cases every year represent a combination of dormant, actively replicating, and drug-resistant Mtb. The global rise of drug resistance necessitates finding new drugs, a slow and expensive process, or repurposing existing drugs. We have been inspired by the latter approach. For example, beta-lactams are extremely effective antibiotics that targets enzymes involved in cell wall biosynthesis. They are safe, cheap, and available globally—but are almost never used to treat TB. However, there have been occasional reports of successfully treating mycobacterial infections with beta-lactam antibiotics, such as a combination of clavulanate with meropenem. In one example, this combination provided long-term cure in 83% of treated patients with extensively-drug resistant TB. The objective of our research has been to use chemical probes to accelerate discoveries in Mtb. We focused our efforts on analyzing two classes of enzymes: penicillin-binding proteins (PBPs) and L,D-transpeptidases (LDTs). PBPs and LDTs work concurrently to maintain the structure and rigidity of the mycobacterial cell wall. Older classes of beta-lactam drugs inhibit the PBPs, but LDTs are most effectively inhibited by carbapenems. In published work, we described a small set of fluorescent beta-lactam probes, including a red fluorescent meropenem. We are using these beta-lactam probes to decipher the molecular targets of beta-lactam antibiotics in Mtb. First, we discovered that various transpeptidases (PBPs and LDTs) are inhibited by carbapenems and that enzyme activity is differentially regulated in dormant and replicating Mtb. However, many of the putative beta-lactam targets were not identified in our analysis. As a result, we have optimized new methods for labeling and enriching beta-lactam drug targets from Mtb lysates for identification by mass spectrometry-based proteomics. We have identified known and putative targets of meropenem. These studies have been enabled by a chemical probe (i.e., biotinylated meropenem). The focus of this presentation will be on our latest findings on the targets of beta-lactam antibiotics in replicating Mtb and in nutrient starvation-induced dormant Mtb.

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Abstract 2481

Protein Synthesis Inhibition Interaction with the aUPR and Autophagy

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Estrogen is a hormone that binds to estrogen receptor alpha (ERα) and promotes growth in ER α positive cancers. BHPI is a biomodulator of ERα and activates a lethal stress pathway called the anticipatory unfolded protein response (aUPR). This pathway leads to cellular necrosis, which involves disruption of calcium homeostasis, cell swelling, ATP depletion, and loss of plasma membrane integrity. A dramatic consequence of BHPI activation of the aUPR is near-complete inhibition of protein synthesis in cells. BHPI has also been shown to inhibit autophagy, which is a process of cells recycling unnecessary or damaged parts. We therefore focused on the relationship between BHPI activation of the aUPR and inhibition of autophagy, in particular, the role that protein synthesis inhibition might play in interfacing between the aUPR and autophagy. To monitor autophagy, Beclin-1 and LC3 were visualized via western blotting. Follow-on studies will further clarify the importance of near qualitative protein synthesis inhibition on possible interactions between the aUPR and autophagy.

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Abstract 2483

Rac and Cdc42 Inhibitors in Rheumatoid Arthritis Therapy

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Anamaris Torres-Sanchez, Patricia Sanchez-Orive, Suranganie Dharmawardhane

Rheumatoid arthritis (RA) is the most common autoimmune disorder that affects connective tissue and joints. Fibroblast synoviocyte proliferation, leukocyte extravasation and neovascularization are common characteristics of this disorder. These processes have been shown to be under the regulation of key signaling proteins Rac and Cdc42 that are central to fibroblast and leukocyte migration during inflammation. Therefore, our objective is to demonstrate the therapeutic effect of our patented Rac/Cdc42 inhibitors MBQ-167 and derivatives in inflammatory cells *in vitro*. The hypothesis is that MBQ-167 and derivatives will block pro-inflammatory mediators in leukocytes and fibroblasts, thus suppressing multiple mechanisms implicated in RA. To test this hypothesis, we determined the effect of Rac activation on synovial cells by Rac.GTP pulldown assays. Using the SW-982 synovial cell line, we demonstrated that MBQ-167 and derivative MBQ-168 inhibit Rac activation. The total Rac levels were also decreased at 250 nM and 500 nM of MBQ-167. Next, synovial fibroblasts were treated with the Rac/Cdc42 inhibitors and tested for cell viability using a MTT assay. Both, MBQ-167 and MBQ-168 decreased cell viability in synovial fibroblasts with a GI50 of 523 nM and 214 nM respectively. Cell migration regulated by Rac is crucial for the inflammatory response in synovial joints, therefore, we tested the effect of Rac/Cdc42 inhibitors in migration by a wound healing assay. Incubation with MBQ-167 and MBQ-168 inhibited more than 50% of synovial cell migration using concentrations from 150 nM for MBQ-167 and 500 nm for MBQ-168. In conclusion, our drugs have the potential to reduce inflammation at arthritic joints not only by reducing macrophage-like cell activation and migration, as shown by initial studies in macrophages, but also synovial fibroblast viability and migration to arthritic joints. Therefore, our Rac/CDC42 inhibitors have the potential to reduce fibroblast and leukocyte migration and inflammation at arthritic joints.

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Abstract 2504

Cytochrome P450 3A4 Suppression by Epimedium Can enhance Anti-inflammatory Effect of Corticosteroid

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Ke Li, Xiu-Hua Yu, Ibrahim Musa, Nan Yang, Xiu-Min Li

Corticosteroids (CS) are the most widely used anti-inflammatory medication and the first line of therapy for a variety of chronic inflammatory autoimmune and allergic disorders. While extremely efficacious, prolonged use of CS is problematic due to the high risk of serious adverse effects. Cytochrome P450 (CYP) 3A4 is a major drug metabolizing enzyme for corticosteroids (CS). Epimedium has been used for asthma and variety of inflammatory conditions with or without CS. It is unknown whether epimedium has an effect on CYP 3A4 and how it interacts with CS. In this study we sought to determine the effects of epimedium on CYP3A4 and whether it affects the anti-inflammatory function of CS. We used RT-PCR and western blotting to determine the effect of epimedium on CYP3A4 mRNA and protein expression respectively in a human hepatocyte carcinoma (HepG2) cell with or without epimedium, dexamethasone, rifampin, and ketoconazole. Epimedium inhibited the expression of CYP3A4 mRNA and further suppressed dexamethasone enhancement of CYP3A4 mRNA expression in HepG2 cells ($p < 0.05$). Epimedium inhibited the protein expression of CYP3A4 ($p < 0.05$). In this study we showed that epimedium has an inhibitory effect on CYP3A4, a corticosteroid drug metabolizing enzyme, which can affect the metabolism and bioavailability of corticosteroids, potentially enhancing its anti-inflammatory effect. The result of this study warrants further *in vitro* and clinical investigation to fully understand the potential mechanisms and by which epimedium mediates it effect. Epimedium might be a potential combination therapeutic option that can enhance anti-inflammatory effect of corticosteroids ensuring appropriate dosing for long-term corticosteroid use.

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Abstract 2506

Xenobiotics, Chlorpyrifos and Tetracycline, degradation by fungal exudates

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John Holliday, Omoanghe Isikhuemhen

Organophosphate, Chlorpyrifos and Tetracycline are well known for their extensive use in aquaculture, field crops and livestock production, especially in diseases and insects pest management. However, the intensive use of these xenobiotic cause environmental pollution in water and soil due to their high toxicity and prolonged persistence in the environment. They are considered a major threat to human beings because they have found their ways in every segment of the environment, persist and cause damage to the ecology and ecosystems they are present, hence the need to remediate them and biodegradation using suitable organisms has become a subject of huge interest today. This study investigated the ability of fungal exudate to degrade Chlorpyrifos (MW. 350.6) and Tetracycline (MW. 444.4) in aqueous media. The exudate was generated by the cultivation of Psathyrella candollena in liquid medium (250 mL) containing potato dextrose broth. After 12 days of incubation at room temperature, the cultivation was terminated and centrifuged to remove the mycelia and the resulting broth used as exudate for xenobiotic degradation. Mixtures containing 20 mL exudate and 20 mL xenobiotic (total concentration 600 ppm) were made and incubated for 24 hrs at room temperature before use in HPLC analyses. Results from the analyses indicated 91.66% and 97.56% decrease in chlorpyrifos and tetracycline concentrations, respectively. Similarly, mixture containing, tetracycline concentration of 20 ppm, indicated 60.40% decrease. These promising results suggest the possibility that biodegradation with fungi could be considered in the remediation of xenobiotic like chlorpyrifos and tetracycline in systems and environments they are found as pollutants.

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Abstract 2525

Spatiotemporal control of cellular cAMP signaling by GPCRs

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Emily Blythe

I will discuss recent developments in our understanding of signaling mediated by G protein-coupled receptors (GPCRs) from distinct membrane locations, focusing on signaling through the G protein-coupled cyclic AMP (cAMP) cascade as an example and comparing the effects of signaling initiated from endosomes to signaling initiated from the plasma membrane. Methods include protein biochemical and cellular imaging approaches to analyze signaling events in intact cells in a spatially and temporally resolved manner. Results will focus on the signaling effects of the vasoactive intestinal receptor 1 (VIPR1) when activated at a native level of receptor expression and in intact, living cells. The VIPR1 reveals a discrete cellular mode of endosomal GPCR signaling, as defined by β-arrestin not being required whatsoever to initiate the endosome-localized component of cAMP cascade activation, and it also delineates a discrete function of β-arrestin in 'sculpting' the spatiotemporal profile of the cell's integrated cAMP activation response in a precise and receptor-specific manner.

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Abstract 2534

Evaluation of the antiglycation effect and AGEs-protein cross-link breakage ability of cinnamon stem-bark water extract

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Motetelo Mogale, Lesibana Sethoga, Sechene Gololo, Leshweni Shai

Advanced glycation end-products (AGEs) are linked to vascular complications of late onset diabetes mellitus. Cinnamon stem-bark has wide usage as an antidiabetic dietary supplement. Study aimed at evaluating anti-glycation and AGEs-protein cross-link breaking effects of cinnamon stem-bark water extract on major types of AGEs derived from both glucose and fructose, comparing with that of aminoguanidine, as a reference drug. Glucose/Fructose solution was incubated with bovine serum albumin (BSA) in the presence and absence of cinnamon stem-bark water extract or aminoguanidine at 37°C. Spectrofluorometry was used to measure the resultant fluorescent AGEs (FAGEs) while enzyme-linked immunosorbent assay (ELISA) was used to measure the total immunogenic AGEs (TIAGEs) obtained. Cross-link breaking activity was measured by means of ELISA. Percentage anti-glycation activity and cross-link breaking ability were calculated. Standard chemical tests were conducted for phytochemical screening. Gas chromatography mass spectrometry was also employed. Phytochemical screening of cinnamon stem-bark water extract revealed the presence of several important phytochemicals. Cinnamon stem-bark water extract showed a dose-dependent anti-glycation effect against both BSA/glucose and BSA/fructose-derived FAGEs that was higher than that of aminoguanidine. A similar IC50 value of 0.13 mg/mL was recorded for the extract against both FAGEs formation. Also, the extract showed significantly ($p < 0.001$) higher anti-glycation activity against BSA/glucose and BSA/fructose-derived TIAGEs than aminoguanidine. Both cinnamon stem-bark water extract and aminoguanidine showed weak cross-linkage breaking activity selectively on BSA/fructose derived AGEs and no activity on BSA/glucose-derived AGEs. Gas chromatography mass spectrometry enabled the identification of (+)-alpha-tocopherol acetate in the methanol fraction of cinnamon stem-bark water extract. Cinnamon stem-bark showed potential for inhibition of major types of AGE. The observed anti-glycation activity of cinnamon stem-bark could be due to the nature of phytochemicals it possesses. The cinnamon stem-bark appears to be poor in breaking already formed AGEs-protein cross-links.

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Abstract 2537

Design and synthesis of Estrone analogs targeting pancreatic cancer

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Fathi Halaweish, Trevor Ostlund, Kakan Sutradhar

Pancreatic ductal adenocarcinoma (PDAC) is the fourth deadliest cancer in the US, the survivability rates are low, typically 6–12 months, and thus a new treatment is needed. Current drug discovery program in our group reported novel estrone analogs targeting EGFR-MAPK pathway. Our hypothesis that fluoro and amine analogs could specifically target EGFR-MAPK cascade. Design of a series of C2, C4 fluoro and C17 amide analogs respectively was conducted (Figure 1). Molecular modeling study of novel fluoro and amine functional groups was conducted using OpenEye® software. Molecular modeling study revealed a promising analog to be the variant ((2S)-2-amino-N-((8R,9S,13S,14S)-4-fluoro-7,8,9,11,12,13,14,15,16,17-decahydro-3-hydroxy-13-methyl-6H-cyclopenta[a]phenanthren-17-yl)-3-(4-hydroxyphenyl) propanamide) which scored the highest binding affinity for almost all molecular target proteins in the study. The consensus score was a 4 out of 395, noting that scores that are low predict a more favorable interaction with the protein. The C2 fluoro was synthesized with selectflour, an electrophilic fluorinating reagent, directly while the C4 fluoro was alkylated with tertbutyl alcohol on the 2-position followed by the fluorination with selectfluor on the 4 positions, followed by the dealkylation. The C17-amine/amide was synthesized by converting the C17 ketone of Estrone into an oxime using hydroxylamine, and then reduced to an amine followed by an amide formation with the amino acid chloride. The compounds synthesized have been characterized using HNMR, C13-NMR and MS. Antiproliferation activities of novel analogs against pancreatic cell cultures Panc-1 and other cell cultures will be conducted. A study of the mechanism of anti-cancer activity will be presented using cell cycle analysis and western-blot analysis.

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ASBMB

Abstract 2538

Engineering remote control of cells using optical or thermal stimuli

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Naturally-evolved protein sensor modules have provided a wealth of strategies for on-demand, remote control of cell behavior. Light-sensitive proteins have allowed optogenetic control of proteins and cells with unparalled precision in both space and time. However, optical control is less well-suited for use in opaque tissues where light is rapidly scattered. There is growing interest in thermal control as an alternative mode of induction, since temperature can be controlled in tissues at high precision at a depth of ~10 cm. However, few strategies exist to control cells with temperature, and existing strategies focus almost exclusively on transcriptional control. We will describe our efforts to advance both optogenetic and thermogenetic technologies through engineering distinct aspects of a single protein, BcLOV4. BcLOV4 is a fungal photoreceptor that translocates to the plasma membrane in response to blue light and has been adopted for optogenetic control of an array of signaling pathways. We will first describe our finding that, in addition to membrane translocation, BcLOV4 forms protein clusters in a light-dependent manner. Dual translocation and clustering is a unique combination for a photoreceptor, and we show how this combined property can be harnessed for new types of optogenetic control. We additionally found that clustering and translocation are causally linked, allowing us to enhance the photosensitivity of BcLOV4 optogenetic tools by adding intrinsically disordered regions to BcLOV. Separately, we previously reported that BcLOV4 integrates both light and temperature cues to specify membrane localization. We have now engineered variants of BcLOV4 (BcLOV-T) that respond only to temperature and not light, such that the protein is clustered and localized at the membrane at low temperatures (27C) but dissociates with an increase of 3–5C in a reversible fashion. With the aid of new devices to reliably control temperature in individual wells of a 96-well plate, we quantified the thermal responsiveness of BcLOV-T and its variants that we engineered for enhanced membrane binding. As a result, we generated 4 variants with thermal switchpoints between 30 and 40C. These include variants that bind the membrane at 37C but not at 42C, the recognized window for safe tissue heating in mammals. Finally, we embedded BcLOV-T variants in molecular circuits to demonstrate that BcLOV-T is a modular actuator that can be rapidly adapted to control signaling, proteolysis, and nuclear localization with thermal stimuli. We expect that such thermal control of post-translational behavior will be highly advantageous for engineered cells, for example allowing external control of where and when therapeutic cells should migrate or proliferate in the course of a therapy.

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Abstract 2563

Beneficial effects of Furosap® - A novel fenugreek seed extract, in improving lean body mass, fat free mass and serum testosterone level: A randomized, placebo-controlled, double blind clinical investigation

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Anand Swaroop, Manashi Bagchi, Sreejayan Nair

Background: Trigonella foenum-graecum (family Fabaceae), an Indian medicinal plant, has demonstrated an array of health benefits in improving glucose tolerance and insulin sensitivity, cardiopulmonary health, metabolic homeostasis, and anti-inflammatory efficacy, as well as in improving sports, muscle mass, and exercise performance by augmenting serum testosterone level.

Methods: This randomized, double-blind, placebo-controlled, clinical investigation evaluated the safety and beneficial efficacy of Furosap, a novel patented fenugreek seed extract enriched in 20% protodioscin, on broad spectrum safety and exercise performance in forty healthy male athletes (n = 40). Athletes were given either placebo or Furosap capsules (250 mg/day b.i.d) over a period of 12 consecutive weeks. Serum samples from these athletes were assessed to determine serum testosterone level and C-reactive proteins (CRP) at the baseline and end of the 12-weeks of supplementation. Moreover, body fat mass, lean mass, fat mass, fat-free mass, grip strength, upper and lower body strength, maximal graded exercise stress using a digital hand dynamometer, dual-energy X-ray absorptiometry (DEXA), force plate, and treadmill with open-circuit spirometry were determined at the baseline and at the end of 12-weeks of treatment.

Results: Increases in mean lean body mass and fat-free mass were observed in Furosap-supplemented subjects as compared to the placebo samples. Serum testosterone levels were also significantly elevated in Furosap-supplemented subjects. Furthermore, Furosap supplementation demonstrated a trend in lowering the blood pressure during exhaustion. No adverse events were observed.

Conclusion: Taken together, Furosap exhibited significant improvement in lean body mass and serum total testosterone levels, and broad-spectrum safety. Overall, Furosap has significant potential in boosting endurance and promoting sports performance.

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Abstract 2579

Imperata cylindrica Reduced Atherogenic Effect in Rat Models on High Cholesterol Diet

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Suhartati Suhartati, Ira Humairah, Hanik Hidayati, Lynda Hariani

The metabolism and transport of lipids and lipoproteins are crucial contributors to cardiovascular disease (CVD). For many years, lipoprotein (a) (Lp(a)) has been acknowledged as an emerging cardiovascular risk factor. Lp(a) is similar in structure to Low Density Lipoprotein (LDL). A variation of LDL, known as Lp(a), is made up of an LDL-like molecule wherein apo B-100 is tightly linked to apo-a glycoprotein. It has been established that an excessive amount of oxidative stress is linked to a number of cardiovascular illnesses. The purpose of this study was to determine whether Imperata cylindrica extract had any anti-atherogenic effects on Lp(a) and malondialdehyde (MDA) levels in rat models fed a high-cholesterol diet. The imperata cylindrica plant is high in antioxidants and lowers cholesterol. Twenty-eight male albino Wistar rats were separated into four groups: K0, normal diet; K1, high-cholesterol; K2 high-cholesterol diet with Imperata cylindrica extract 5 mg/200 gram Body Weight (gBW); K3, high-cholesterol diet with 15 mg/200 gBW extract of Imperata cylindrica (K3). Lp-a and MDA serum were measured following the intervention period. Rats on a high-cholesterol diet (K1) showed a significantly higher level of blood Lp(a) than the control group (K0). Administration of Imperata cylindrica extract 5 mg/200 gBW can lower levels of Lp(a), but this effect is not statistically significant with $p = 0.392$, but Imperata cylindrica extract 15 mg/200 gBW can significantly with $p = 0.032$ lower levels of Lp(a). However, the level of MDA did not significantly rise ($p = 0.238$). By lowering Lp(a) levels, Imperata cylindrica extract was shown to have anti-atherogenic properties.

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Abstract 2582

Ultra High-Throughput Screening Assays for Inhibitors of TMPRSS2 and SPIKE Interaction

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Qiankun Niu, Kun Qian, Dacheng Fan, Andrey Ivanov, Stefan Sarafianos, Yuhong Du, Haian Fu

SARS-CoV-2, the coronavirus that causes the disease COVID-19, was identified over three years ago, yet current small molecule therapies have limited usefulness and resistance to therapies and vaccines is inevitable. Ultra high-throughput screening (uHTS) assays for novel and repurposed inhibitors of a protein-protein interaction in the viral life cycle could be used to screen a vast number of compounds with a specific target of action. In particular, the interaction between viral SPIKE protein and human TMPRSS2 is an understudied, yet critical step in viral entry. Thus, we aim to create uHTS assays to rapidly and affordably identify inhibitors of the TMPRSS2 and SPIKE interaction for further biochemical studies and therapeutic development for SARS-CoV-2. We first sought to create a Time Resolved-Forster/Fluorescence Energy Transfer (TR-FRET) assay which uses lysates of cells with overexpressed SPIKE and TMPRSS2 and fluorescently labeled antibodies to detect interactions between these proteins. Initially, we developed and optimized this TR-FRET assay in a 384-well plate then miniaturized to a 1536-well plate. We conducted a pilot screen of compounds with known biological activity to test this assay's screening capabilities. To further narrow the hits from this TR-FRET screen, we developed an orthogonal uHTS Nanoluciferase Binary Technology (NanoBiT) assay to detect the interaction between tagged SPIKE and TMPRSS2 in live cells. With these two assays in hand, we expanded our TR-FRET screen to over 100 000 compounds and identified several that were also positive in the orthogonal NanoBiT assay. Four of these compounds were found to potentially interact with either SPIKE or TMPRSS2 from thermal shift experiments, providing support for their action as SPIKE and TMPRSS2 interaction inhibitors. Thus, we have developed TR-FRET and NanoBiT orthogonal uHTS assays which have allowed for the discovery of several possible repurposed and novel inhibitors of the SPIKE/TMPRSS2 interaction. These uHTS assays can be employed as a model for future drug discovery efforts and the compounds identified may be used as exciting starting points for development of inhibitors of SARS-CoV-2.

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ASBMB

Abstract 2583

Advance 19F NMR Conformational Resolution to Map Populations of GPCR Sub-states and Determine Biased Ligand

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G-protein-coupled receptors (GPCRs) constitute the largest protein family of receptors, which can be activated by a range of external factors from small molecules to large proteins. The activation of GPCR will result in conformational change of the receptor in order to transmit the signal to downstream partners such as various G proteins, GRKs, and beta-arrestin, etc. Due to the difficulty of delineating the receptor conformations, our understanding of how the receptor differentially engages various partners remains unclear. The high sensitivity of 19F to the surrounding environment, 100% natural abundance and high sensitivity to NMR detection make 19F NMR spectroscopy useful for protein structure and dynamics study. Discovering sensitive 19F probes is the most direct strategy to improve conformational resolution. Here, we performed large-scale tri-fluorinated compound screening and examined micro-electrostatic sensitivities of tri-fluorinated compounds when conjugated to the receptors. However, residues buried inside the protein's hydrophobic core cannot be labeled through post-translational cysteine-mediated chemical conjugation. To overcome the limitations of the cysteine conjugation, we applied genetic incorporation of trifluoromethyl-L-phenylalanine (tfm-Phe) into A2AR, expressed in Pichia pastoris. We expect that these methods will expand our capacity of probing GPCR for conformational transition and dynamics study.

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Abstract 2605

Microtubule depolymerization by estramustine induces ferroptosis in neuroblastoma cells

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Dulal Panda

Estramustine, a microtubule-targeting compound, has been in clinical use for a long time to treat prostate cancer. In this study, we have explored the anticancer potential of estramustine against human neuroblastoma cells (SH-SY5Y) and elucidated its antiproliferative mechanism. Estramustine inhibited the proliferation of SH-SY5Y cells with a half-maximal inhibitory (IC50) concentration of 11.7 ± 0.8 μM. Estramustine treatment caused the depolymerization of microtubules of SH-SY5Y cells in a concentration-dependent manner. A flow cytometric analysis of the propidium iodide-stained DNA suggested that the compound induced cell cycle arrest in the G2/M phase. Estramustine (24 μM) arrested 73% of the cells in the G2/M phase of the cell cycle after 24 hours of treatment. The cell killing ability of estramustine on SH-SY5Y cells was analyzed by flow cytometry. Estramustine (24 μM) treatment for 48 hours induced 70% cell death in SH-SY5Y cells. These results indicate an underlying role of microtubules in the anti-tumor efficacy of estramustine. Further, estramustine produced reactive oxygen species (ROS) in SH-SY5Y cells in a concentration-dependent manner. Additionally, the pre-treatment of SH-SY5Y cells with N-acetyl cysteine, a scavenger of ROS, significantly abrogated the antiproliferative effects of estramustine. The result indicates a possible role of ROS in the anti-tumor mechanism of estramustine. Further, estramustine induced lipid peroxidation, a hallmark feature of ferroptosis, suggesting the possibility of induction of ferroptotic cell death. A ferroptosis inhibitor, deferoxamine mesylate, significantly abrogated the cytotoxic effects of estramustine, thus supporting the role of ferroptosis in the anti-tumor activity of estramustine. Interestingly, the pre-treatment of cells with deferoxamine mesylate did not affect estramustine-induced depolymerization of microtubules. The results suggested that estramustine induced ferroptotic cell death in neuroblastoma cells via microtubule depolymerization.

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Abstract 2635

Targeting virus-induced vulnerabilities using synthetic lethality as a new class of host-based antivirals

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RNA viruses are the major class of human pathogens responsible for many global health crises, including the COVID-19 pandemic. However, the current repertoire of U.S. Food and Drug Administration (FDA)-approved antivirals is limited to only nine out of the known 214 human-infecting RNA viruses, and almost all these antivirals target viral proteins. Traditional antiviral development generally proceeds in a virus-centric fashion, and successful therapies tend to be only marginally effective as monotherapies, due to dose-limiting toxicity and the rapid emergence of drug resistance. Host-based antivirals have potential to alleviate these shortcomings, but do not typically discriminate between infected and uninfected cells, thus eliciting unintended effects. In infected cells where host proteins are repurposed by a virus, normal host protein functions are compromised; a situation analogous to a loss-of-function mutation, and cells harboring the hypomorph have unique vulnerabilities. As well-established in model systems and in cancer therapeutics, these uniquely vulnerable cells can be selectively killed by a drug that inhibits a functionally redundant protein. This is the foundation of synthetic lethality (SL). To test if viral induced vulnerabilities can be exploited for viral therapeutics, we selectively targeted synthetic lethal partners of GBF1, a Golgi membrane protein and a critical host factor for many RNA viruses including poliovirus, Coxsackievirus, Dengue, Hepatitis C and E virus, and Ebola virus. GBF1 becomes a hypomorph upon interaction with the poliovirus protein 3A. A genome-wide chemogenomic CRISPR screen identified synthetic lethal partners of GBF1 and revealed ARF1 as the top hit. Disruption of ARF1, selectively killed cells that synthesize poliovirus 3A alone or in the context of a poliovirus replicon. Combining 3A expression with sub-lethal amounts of GCA – a specific inhibitor of GBF1 further exacerbated the GBF1-ARF1 SL effect. Together our data demonstrate proof of concept for host-based SL targeting of viral infection. We are currently testing all druggable synthetic lethal partners of GBF1 from our chemogenomic CRISPR-screen, in the context of dengue virus infection for their abilities to selectively kill infected cells and inhibit viral replication and infection. Importantly, these SL gene partners of viral-induced hypomorphs only become essential in infected cells and in principle, targeting them will have minimal effects on uninfected cells. Our strategy to target SL interactions of the viral-induced hypomorph has the potential to change the current paradigm for host-based therapeutics that can lead to broad-spectrum antivirals and can be applied to other intracellular pathogens.

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ASBMB

Abstract 2646

The effect of Chinese Traditional Medicine Herbal Decoction Tea on respiratory allergy profile region XVII and possibility of affecting the liver as seen in respiratory allergy (IgE) reaction as well as in the Aspartate Aminotransferase (AST) and Alanine

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Objective: The goal of this study was to analyze and show the effect of Chinese Traditional Medicine Herbal Decoction Tea on respiratory allergy profile region XVII and possibility of affecting the liver function as seen in the levels of respiratory allergic specific (IgE) reaction as well as noticed from (AST) and (ALT) enzymes levels.

Methods: A study of an out-patient of an African origin in the San Francisco Bay Area with a chief complaint of pre-diabetic problem initially with hemoglobin A1c at 7.4% level, glucose level of 110 mg/dL, AST 31 U/L and ALT 28 U/L (normal levels) as of April 2017 was conduct from April 2017 through June 2022, (a five-year study). In the beginning of 2018, the patient started receiving Chinese Traditional Medicine (CTM), specifically Herbal Decoction (tea) with a dosage of 3–6 times daily after brewing it. In 2019 the patient was discontinued from CTM to see whether there was any change in AST and ALT and allergy (IgE) levels. At the end of 2020 through 2021 the patient was put back on CTM to see whether there was any effect on AST and ALT as well as (IgE) levels. The analysis and observation of the patient's AST and ALT enzymes' behavior during the five-year period was done. Three laboratory respiratory allergy profile region XVII tests spaced from 2017, 2018, 2021 and 2022 were done.

Medical History: Patient was 172 lbs, 42 years of age, never smoked in his life, never had drugs abuse in his life, never drunk alcohol, was not sexually active. Worked in finance department.

Results: After a detailed review, it was seen that the levels of AST, ALT rose, and the level of immunoglobulin E (lgE) increased too to 1333 kU/L by 2021 when the patient was on (CTM) herbal decoction tea treatment. The AST and ALT levels decreased after discontinuing the patient from treatment while E (IgE) levels continued to rise. The patient also developed a reactive result to hepatitis B, AB total and Hepatitis A, AB total in August 2019 and April 2020 respectively.

Conclusion: Although there could be other factors affecting the health of this patient, the study suggested that CTM herbal decoction tea was the underlying factor in this case that causes AST and ALT enzymes to go up due to herbal decoction tea that the liver was trying to get rid of out of the system as a drug toxicity, hence making the patient react to hepatitis problems. However, the comprehensive composition of CTM herbal decoction tea as to determine which individual herbals are in the decoction remains a puzzle to solve in discovering which exactly herbal(s) truly causes hepatitis problem. Further complications were seen in the patient as the patient developed Allergic Rhinitis (AR) as respiratory allergies rampaged on this patient especially allergies to tree groupings, weed groupings and cockroaches as indicators of these groupings were increasing in levels. The level of immunoglobulin E (lge) was seen to be increasing up to 1333 kU/L by 2021. Patient became allergic to food stuffs such as peanut, wheat, soya beans, sesame, shrimp, hazelnut, almond and scallop showing specific allergens or lge. This knowledge is important because it will help create such a curative therapeutic model for liver problems and respiratory allergies by which scientists may find it a beneficial combination attribute to other existing therapeutic regimes to cure respiratory allergies and reduce liver problems. Further clinical study should be done on the allergic rhinitis (AR) that is being worsened by CTM. As a result of the above complications, patient has been now put on a long term Allergen Immunotherapy Treatment (AIT) and Xolair at the hospital. The results show improvement.

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Abstract 2657

SNAP-TurboID: A Proximity-based Intracellular Tool for Small Molecule Target Identification

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Introduction: Drug discovery campaigns often begin with the use of a phenotypic assay to screen a library of compounds for a therapeutic response. One major advantage of phenotypic screens is their ability to elucidate different therapeutic modalities; however, by just measuring a cellular response, each hit requires a separate target identification study to discern mechanism of action. Current approaches to target deconvolution include affinity purification, reactive covalent probes, thermal proteome profiling, and activity probes, but all these methods have limitations including scalability and propensity to yield false negatives. To address the challenges posed by existing mechanism of action studies, we pursued a novel target identification approach using SNAP-TurboID, an intracellular proximity labeling technology utilizing a highly active biotin ligase (TurboID). SNAP-TurboID consists of TurboID linked via a variable GGGGS linker to a SNAP tag—a protein with an accessible cysteine capable of forming a covalent link to a benzylguanine moiety. In practice, small molecule hits identified via phenotypic screening can be modified with a benzylguanine moiety, thereby allowing SNAP-TurboID to complex with the small molecule and biotinylate proteins in the complex's direct vicinity. Biotin affinity chromatography can then be used with a variety of proteomic processing methods and LC-MS/MS to analyze enriched protein species or biotin-modified peptides in the bound fraction for clues to the compound's mechanism of action. A proof-of-concept study employed methotrexate modified with a benzylguanine using several different linker designs for characterization and development of SNAP-TurboID.

Methods: Plasmids encoding SNAP-TurboID were designed using a pcDNA3.1 backbone. SNAP-Turbo constructs that would be capable of detecting small molecule interactions within specific subcellular compartments were designed by appending localization sequences that direct the fusion protein to the cytoplasm, endoplasmic reticulum, Golgi, peroxisome, mitochondria, plasma membrane, nucleus, nucleolus, or extracellular environments. The SNAP-Turbo proteins were transiently expressed in HEK293T using Lipofectamine 2000 transfections. Biotinylated proteins were captured using streptavidin beads and protein identification was performed using immunoblots or LC-MS/MS.

Results: The bifunctional SNAP-TurboID protein retained its ability to biotinylate and conjugate with SNAP substrates. Optimization experiments demonstrated that the labeling ability of SNAP-TurboID was dependent on both time and biotin concentration. Additionally, when TurboID was expressed at high levels using a strong promoter, we observed excessive labeling that precluded our ability to define the small molecule interactome. Our findings point to optimization of transfection conditions, the importance of promoter choice, and development of stable cell lines as important steps in optimizing SNAP-TurboID's labeling performance and reproducibility within cells.

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ASBMB

Abstract 2658

Characterizing the Mechanism of Resistance of PRC1590 Using Known Antimalarials

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Malaria is a disease caused by the Plasmodium parasite, with the deadliest species being *Plasmodium falciparum*. In 2021 there were 247 million cases of malaria reported worldwide. Resistance to all available antimalarial drugs has been identified in regions endemic to this disease. As a result, it is imperative to find novel antimalarials with new molecular targets, and to better understand common mechanisms of resistance to the known antimalarials. Mutations in the multidrug resistance protein (PfMDR1) can lead to collateral drug sensitivity, which is when the parasite becomes resistant to certain compounds, but more sensitive to others. This is important to study because it can inform partner drug pairing and lessen failed attempts for treatment due to antimalarial resistance. PRC 1590 is a potential antimalarial compound discovered by our lab. This compound has a monophasic curve and a low nanomolar EC50 (half maximal effective concentration). The EC50 measures the concentration of a given drug that would be required to induce half of the maximum possible effect. A lower EC50 is necessary when searching for prospective antimalarial compounds. Through early characterization of this compound, we identified that the mechanism of resistance is due to a single nonsynonymous mutation on the pfmdr1 gene. One of the first steps in antimalarial discovery is to generate *in vivo* resistance to compounds. Previously, the lab was able to select for a PRC1590-resistant strain of parasites using the drug-sensitive 3D7 strain. Single nucleotide polymorphisms (SNPs) and gene amplification mutations such as copy number variants (CNVs) lead to resistance to antimalarials. PfMDR1 is an ATP- binding cassette transporter. These types of transporters are frequently associated with multidrug resistance. This pump transports products into the digestive vacuole of the parasite, an organelle similar to the lysosome in humans. In order to understand the mechanism of resistance to PRC1590, we conducted cross-resistance screenings using compounds that have known mechanisms of resistance. Currently, we are conducting assays to continue analyzing the characteristics of three different strains when exposed to known antimalarials such as amodiaquine, quinine, chloroquine, and mefloquine. Our results have shown that 3D7- 1590 resistant strains present collateral drug sensitivity to mefloquine and quinine due to demonstrated resistance to PRC1590. 3D7 and 3D7-1590 resistant strains presented similar EC50 values when treated with chloroquine, quinine, and amodiaquine. Our results are setting the basis for future studies to understand how antimalarials may select for mutations in PfMDR1.

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Abstract 2665

Targeting human oncogene PKC iota in various cancers through PROTAC targeting degradation

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The protein kinase C (PKC) family of serine/threonine kinases were linked to tumor promoting activities over 50 years ago and have been studied immensely to understand the mechanisms of action and associated pathways of each isozyme. This family of enzymes consists of the conventional isoforms, which contain DAG and calcium (C2) binding domains, the novel isoforms which only contain the DAG domain, and the atypical isoforms that have neither a C2 or functional DAG domain, rather activation is dependent upon protein interactions mediated by the PB1 domain. PKCι is an atypical isoform that has been characterized as an amplified driver kinase in many cancers, with amplifications of its gene PRKCI in lung squamous cell carcinoma (SCC) (36%), ovarian serous carcinoma (23%), esophageal adenocarcinoma (19%), and head and neck SCC (14%). PKCι is involved in several signaling pathways implicated in cancer, including cellular transformation (Bcr-Abl/Ras), tumor growth (MEK/ERK), and survival/chemoresistance (NF-KB). PKCι can regulate tumorigenic processes via mechanisms dependent and independent of catalytic activity, including protein protein interactions with par6, zip/p62, and MEK5. Therefore, a degrader has great potential as a novel therapeutic to treat cancers of unmet need harboring amplifications in PKCι to target both catalytic and non-catalytic mechanisms of tumorigenesis regulated by PKCι. In collaboration with Rolf Swenson, Ph.D. at NHLBI, we developed an initial set of degraders using compound 49, an inhibitor of PKCι developed by Kwaitkowski, et al. to tether to a ligand for VHL E3 ligase. Initially, we will overexpress PKCι in HEK293T and HeLa cells to treat and probe the effects of compound 49 and PROTACs via western blot. We aim to transition our work to cancer cell lines with amplificied PRKCi. This will allow us to better understand the efficacy of PROTACs in tumor cells and focus on elucidating mechanisms by which PKCι is promoting tumorigenesis independent of its catalytic activity by comparing PROTACs with compound 49, and identify pathways impacted by PKCι degradation alone. To further validate the effects of our compounds, we will test our PROTACs via gold standard approaches including using epimers that do not bind E3s, inhibiting the proteasome and neddylation, processes essential for ubiquitin mediated degradation. Ultimately, we will take our most promising hit forward to invivo studies (PDX and cell line xenograft models) to understand the efficacy of our top PROTAC against tumor models. We have successfully expressed PKCι in HEK293T and HeLa cells and the initial treatments with compound 49 and PROTAC 1 show no degradation of PKCι. However, we hope to continue treating transfected HEK239T and HeLa cells with the remaining PROTACs (2, 3, and 4) to compare efficacy *in vitro* and use this information to improve and develop additional PROTACs. PKCι is overexpressed in many human cancers. The significance of PKCι in tumorigenesis via its catalytic dependent and independent roles make it a promising target for development of PROTACs. We aim to develop PKCι-targeting PROTACs, to characterize the effects of inhibiting and degrading PKCι on downstream pathways, as well as understand the efficacy of our top hit in PDX and tumor xenograft models.

We would like to thank the NIH for their continuous support of our research.

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ASBMB

Abstract 2666

Reversing breast cancer resistance protein, BCRP-mediated multidrug resistance *in-vitro*

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Chemotherapy is one of the most effective methods for treating metastatic cancers. Despite the advances in chemotherapy, multidrug resistance (MDR) remains a major obstacle to successful therapeutic outcomes of the treatments. Overexpression of ATP - binding cassette (ABC) transporters like Breast Cancer Resistance Protein (BCRP) in cancer cells lead to multi-drug resistance (MDR) in which cancer cells develop tolerance for multiple structurally or functionally different therapeutic agents. Clinically approved BCRP inhibitors therefore remain an important and unmet medical need. Using computational methods, we have identified new drug-like compounds that are predicted to inhibit BCRP protein pumps. To assess these inhibitors for their ability to block BCRP action, it is important to identify MDR cancer cell lines that overexpress BCRP at clinically relevant levels. For this reason, different MDR cell lines derived from the breast cancer cell lines, MCF - 7, were generated that over express BCRP at different levels. Resistance to chemotherapeutics was assessed and compared to the level of protein expression. Co-administration of the experimental inhibitors with the chemotherapeutic drugs, mitoxantrone and SN38, led to decreased cell viability. Colony formation was also decreased in this BCRP overexpressing breast cancer cell line. We show that the intracellular retention of transport substrates of BCRP is enhanced in the presence of these inhibitors. Our results support the hypothesis that combined treatment of the MDR cancer cells with chemotherapeutics and the newly identified inhibitors may reverse MDR and decrease cell viability and proliferation by increasing intracellular accumulation of chemotherapeutics that are transport substrates of BCRP.

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Abstract 2667

Targeting Telomeric and c-myc G4 DNA as an Anticancer Approach

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G-quadruplex (G4) DNA are non-canonical higher order DNA structures formed from guanine rich sequences, made up of stacked G-tetrads (a cyclic array of four guanine bases) stabilized by Hoogsteen base pairing and K+ ions. G4 DNA is formed in both telomeric sequences at the end of our chromosomes as well as within the promoter region of oncogenes (such as the c-myc oncogene). Initial interest in G4 revolved around its presence in telomeric DNA, as this G4 structure was found to inhibit the enzyme telomerase, which is overexpressed in more than 90% of cancer cells. More recently, G4 has been shown to be overrepresented within the c-myc oncogene. This oncogene regulates cell proliferation, differentiation, and apoptosis The c-myc oncogene is associated with at least 20% of human cancers. Thus, G4 DNA represents a viable target for possible anticancer therapeutic agents to treat previously "undruggable" regions, such as the c-myc oncogene. In this work, G4 structures formed at both telomeric and c-myc G4 sequences were investigated, with an emphasis on c-myc G4. This was done by probing the G4 structures with a variety of known and novel compounds such as quinacrine, TMPyP4 and NDI derivatives. Using the biophysical techniques of isothermal titration calorimetry (ITC), fluorescent displacement, thermal melting, and circular dichroism (CD) spectroscopy, the binding characteristics of these compounds to G4 DNA were investigated. Preliminary studies show that the compounds of this study bind to G4 DNA with high affinity (~107 M-1), with both quinacrine and TMPyP4 displaying multiple binding sites (~107 and 106 M-1), while enhancing the stability of the G4 DNA by increasing its Tm. All binding events were enthalpically driven with strong exothermic peaks. CD was used to confirm that the telomeric and c-myc G4 structures were in a hybrid/antiparallel and parallel conformation, respectively. These findings are encouraging and have implications in targeting these G4 DNA as an efficacious anti-cancer strategy.

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Abstract 2693

Shikonin and alkannin induced inhibition of *Escherichia coli* ATP synthase

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Megan Watson, Timoteea Saitis

Background: ATP synthase, the fifth complex in the electron transport chain, is the main source of ATP in almost all organisms from bacteria to man. Malfunction or inhibition of ATP synthase results in lower levels of ATP available for metabolism, leading to the cell death. A wide variety of inhibitors are known to bind and inhibit ATP synthase. Shikonin and alkannin are known for their antioxidant, anticancer, antiinflammatory, and antimicrobial properties. Here we examined whether the antimicrobial properties of shikonin and alkannin can be linked to the selective inhibition of ATP synthase using *Escherichia coli* as a model system.

Methods: Comparative wild-type, mutant, and null *E. coli* growths were evaluated on limiting glucose and succinate media at OD595. ATP synthase was isolated from wild type and mutant *E. coli* strains by growing them on minimal media to late log phase. Cells were harvested, French Pressed, and passed through ultracentrifugation to isolate and purify membrane-bound F1FO ATP synthase. The null control was *E. coli* pUC118 with deleted ATPase gene. The wild-type and mutant ATP synthase samples were inhibited in presence of varied concentrations of shikonin and alkannin at room temperature.

Results: The protein purity and integrity were confirmed by anti-F1-β antibody immunoblotting. Shikonin and alkannin fully inhibited wild type while variable degree of inhibition was observed for the mutant ATP synthase.

Conclusions: Potent inhibition of wild type ATP synthase by shikonin and alkannin implies that antimicrobial properties of shikonin and alkannin can be linked to the selective inhibition of ATP synthase.

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Abstract 2695

Enzymatic Uncaging Systems for Optically Measuring Membrane Potential

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Deshka Neill, Caroline Christophersen, Nelson Gaillard, Evan Miller

Changes in membrane potential drive cellular processes and interactions across a variety of cell types, including cardiomyocytes and neurons. These rapid changes in electrochemical potential are challenging to study. Optical measurements of membrane potential (Vm) allow for non-invasive, high throughput methods to probe electrophysiology and better elucidate these cellular interactions. The Miller lab has previously developed voltage sensitive fluorophores, or VoltageFluor (VF) dyes. While useful, two substantial limitations of VF dyes are low water solubility and inability to label specific cells types in a co-culture or complex biological system. To address these limitations, my work aims to develop enzymatic uncaging targeting systems for localizing VF dyes to specific cells populations in complex biological systems. This targeting strategy allows us to combine the sensitivity and brightness of chemically-synthesized dyes with the specificity of genetically-encoded proteins. Previous work employed an esterase enzyme to locally cleave a bulky cyclopropyl methyl ester-caged VF dye to activate the fluorophore in cells and brain slices. However, this system is limited because of low water solubility and off-target uncaging by endogenous esterases. My current enzymatic uncaging system of interest utilizes β-glycosidases to remove a β-linked sugar modification from a VF dye analog. This modification aids in increasing the water solubility of our VF substrate and the β-glycosidase improves orthogonality in mammalian systems, as it is derived from bacteria or yeast. Currently, three enzymes have been expressed on the cell surface and are being characterized for substrate compatibility and kinetics. We expect to be able to measure enzyme kinetics with a variety of commercially available substrates, then utilize the systems with the paired caged-VF dye in cells to track individual neuronal action potentials. We anticipate these β-glycosidases to further improve upon our previous esterase method for tracking single cell membrane potential changes in complex systems. Future work aims to multiplex several orthogonal uncaging enzymes and caged VF substrates to track membrane potential changes across numerous populations of cells in real time. This work will enable multi-color imaging to contribute to a growing field of understanding and ultimately manipulating neuronal circuit interactions for therapeutic development.

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ASBMB

Abstract 2708

Imaging Macrophage Polarization with Bioluminescent Reporters and Spectral Phasor Analysis

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Bioluminescence spectral phasor analysis macrophage polarization gene expression reporters BRET.

Macrophages are intricately involved in tissue development and repair. These cells exhibit a spectrum of behaviors upon activation, and are generally classified as one of two types: inflammatory (M1) or anti-inflammatory (M2). M1 macrophages are typically associated with pathogen killing, while M2 macrophages play central roles in tissue healing and growth. A complete understanding of these phenotypes, along with intermediate behaviors along the M1-M2 axis, requires methods to track dynamic macrophage function. Imaging tools have much to contribute in this regard, and several macrophage reporter cells have been developed. However, many existing cell lines are limited to either *in vitro* model systems or tracking a single marker over time. We are developing bioluminescent macrophage reporters to enable multiplexed imaging *in vivo* over time. Bioluminescence involves light production from luciferase enzymes and luciferin small molecules and is advantageous for serial studies because no excitation light is required. Bioluminescence has historically been limited, though, to tracking single biological features over time due to a lack of distinguishable reporters. Bioluminescent probes generally have large overlapping spectra, preventing facile identification. To address this limitation, we developed bioluminescence resonance energy transfer (BRET) based reporters for imaging macrophage polarization. BRET reporters provide a multicolor readout via fusion of luciferase enzymes to various fluorescent protein acceptors. M1- and M2- specific promoters were used to drive the expression of various BRET reporters in macrophage cell lines. Further resolution was obtained via spectral phasor analysis. This approach transforms the emission data collected in each pixel into spectral phasors. With BRET-based reporters and spectral phasor analysis, we monitored the expression of multiple reporters over time in RAW 264.7 and THP-1 macrophages. Changes in reporter expression were analyzed upon macrophage stimulation with various cytokines. Spectral phasor analysis could readily assign the distinct signals, enabling single-cell imaging of macrophage polarization over time. Ongoing work focuses on expanding the number of M1- and M2- specific reporters in the cell lines and applying the reporter cells in various *in vivo* models. Overall, this work broadens the scope of tools for monitoring macrophage polarization and efforts to dial in desired immune cell behaviors.

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Abstract 2709

Insulin and reef-building corals

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Meghana Murthy, Simon Weinzweig, Paniz Jasbi, Giovanna Ghirlanda, Lenore Cowen, Nastassja Lewinski, Judith Klein-Seetharaman

Reef building corals are keystone organisms of tropical marine ecosystems. Corals are involved in a symbiosis with microscopic endosymbiotic algae. This mutualistic relationship is central to reef growth and coral survival, and fulfills 90% of energetic needs suggesting a transport and signaling system where a molecule like insulin could come into play. The breakdown of symbiosis, commonly referred to as coral bleaching, furthermore shows that this mechanism can be disrupted with potential similarities to the diabetic response in vertebrates. Could coral have diabetes? Insulin production is recognized as an evolutionary ancient function and its presence has been demonstrated from human and eukaryotes, to fungi, worms and fruit flies (Muthukumar and Lenard, 1991). Human insulin has been successfully tested in other organisms suggesting a conservation of structure and function across long evolutionary distances (Baig and Khaleeq, 2020). Here we present a study of the insulin signaling pathway in corals through remote homology detection and modeling (Murthy et al, under review), combined with a pilot-study of coral *in vitro* response to insulin (Roger et al., 2022). Using the insulin-receptor system as a proof-of-concept and docking known insulin receptor ligands to a coral homolog structure, we were able to locate ligand binding pockets and demonstrate their conservation in corals. By developing *in vitro* coral cell culture methods we were able to test the cytotoxicity of insulin for the first time in coral cells. Our findings suggest that insulin reduces cell viability of branching coral Pocillopora damicornis (∼18% decrease) at concentrations between 10 and 100 μg/mL. Insulin cytotoxicity is known to depend on different solvent properties, such as increased temperature and high concentrations of salts leading to insulin aggregates and misfolding. Circular dichroism analysis of insulin suggests that the high ionic strength of seawater could have caused a modification in the conformation of insulin which may contribute to the cytotoxic behaviour. The present work lays the foundation for further research related to remote homology applied to corals by developing a pipeline for establishing the functional similarities between human and coral membrane receptor singling systems. Furthermore this work also lays the foundations for further research into the effects of insulin on corals and potential implications such as the new concept of coral medicine.

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Abstract 2719

ACEREVERS-2: Development and validation of a miniaturized Artificial Biosensor System for Identification and Treatment of Diabetic Ketoacidosis (DKA)

Jayshree Mishra, *Texas A&M University-College Station*

Priyam Kumar, Narendra Kumar

Type-1 Diabetes (T1D) affects around 1.6 million (CDC). Diabetic Ketoacidosis (DKA) accounts for almost 56–74% of T1D-induced mortality. During the past decade, DKA-admission rates have increased exponentially with a third of hospitalizations resulting in multiple readmissions within the year of initial treatment. Statistics indicate many of these visits are unplanned, emergent, and critically severe. The DKA Prototype Project: ACEREVERS-2 (a latin for "acid turning") is designed to provide immediate, responsive treatment to ketoacidotic effects caused by cellular inability to ingest glucose by utilizing electronics-based bioengineering and chemical techniques. First, the project created a prototype consisting of a Ketoacidotic receptor able to conduct a current in an acidic solution simulating the blood conditions during DKA. The prototype's ability to run the circuit during such a condition by activating a peristaltic pump that transfers an electrolytic solution consisting of NaHCO3 and insulin into the system containing one of a major ketone body, 3-hydroxybutryate, mixed with acetic-acid. By adjusting the potentiometers to specific resistances that allow ACEREVERS-1 to recognize and inhibit the circuit at neutral pH of 7.0–7.4, the pump can transfer the bicarbonate/insulin until the acidic effects are neutralized. Further improvement checkpoints include adding a heat-resistant casing, cooling measures to offset overheating of the MOSFET, minimizing the circuit and sensor into a series of non-invasive microarrays simulating other pancreatic functions such as infusion of glucagon and/or somatostatin based on the blood conditions needed only a 100 microliters blood serum to diagnose and prevent DKA induced. The miniaturized version of the sensor ACEREVERS-2 device was tested in laboratory conditions with buffer and in simulated clinical analysis with mouse and human blood serum. The results show that this biosensor is capable of DKA diagnosis. Future goal is to further miniaturize the sensor and connected to the SMART phone which can use a drop of blood to detect the DKA.

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Abstract 2726

Biophysical and In-Silico Investigation of Polyphenols Targeting Bifunctional DAH7PS from *Bacillus subtilis*

Anchal Sharma, *University of Illinois at Chicago*

Vijay Kumar, Neetu Neetu, Pravindra Kumar

Antimicrobial resistance (AMR) is currently one of the greatest threats to public health worldwide, so identification and development of novel and broadly effective antimicrobial agents has become an urgent priority. Natural products such as polyphenols, alkaloids, sulfur-containing phytochemicals, and terpenoids etc., have proven as the effective tool for the discovery of new drugs. Cholorogenic acids (CHL), a polyphenol, has demonstrated the potential antibacterial activity by inhibiting enzymes of essential metabolic pathways and other mechanisms. The shikimate pathway synthesizes the essential aromatic amino acids in bacteria, parasites and plants and absent in the higher organism. Thus, the enzymes of this pathway are attractive potential drug targets. Interestingly, our previous structural and functional studies have evidently shown that CHL exhibits antibacterial activity and targets the different enzymes of the shikimate pathway. In the present study, we have characterized DAH7PS the first committed enzyme of the shikimate pathway, from *Bacillus Subtilis* (BsDAH7PS). More importantly, we investigated the binding features of BsDAH7PS with the CHL and polyphenols (Prephenate, Ferulic acid, and Shikimate) using biochemical, biophysical and in silico-based methods. The inhibition studies have demonstrated that CHL non-competitively inhibits the BsDAH7PS activity and suppress the growth of *B. subtilis* (MIC ∼30–40 ug/ml). The CD and fluorescence spectroscopic results have suggested that binding of the polyphenols with BsDAH7PS enhanced the helical content and reduced the flexibility of the protein during the interactions. The possible quenching mechanism is found to be static with Kq values higher than 1010, which is associated with the ground state complex formation of polyphenols with BsDAH7PS. Subsequently, the thermodynamic data revealed that binding of these polyphenols with BsDAH7PS is exothermic and spontaneous in nature. The molecular docking analysis has revealed that CHL have shown the maximum binding affinity (binding energy −8.0 kcalmol-1) and making contacts via hydrogen and hydrophobic interactions with BsDAH7PS. Moreover, in the future designing and optimization of more promising analog compounds based on this scaffold will be helpful to obtained multi-targeted antibacterial agents.

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Abstract 2736

Von Hippel–Lindau (VHL) small-molecule inhibitor binding increases stability and intracellular levels of VHL protein

Julianty Frost, *University of Liverpool*

Sonia Rocha, Alessio Ciulli

Von Hippel–Lindau (VHL) disease is characterized by frequent mutation of VHL protein, a tumor suppressor that functions as the substrate recognition subunit of a Cullin2 RING E3 ligase complex (CRL2VHL). CRL2VHL plays important roles in oxygen sensing by targeting hypoxia-inducible factor-alpha (HIF-α) subunits for ubiquitination and degradation. VHL is also commonly hijacked by bifunctional molecules such as proteolysis-targeting chimeras to induce degradation of target molecules. We previously reported the design and characterization of VHL inhibitors VH032 and VH298 that block the VHL:HIF-α interaction, activate the HIF transcription factor, and induce a hypoxic response, which can be beneficial to treat anemia and mitochondrial diseases. How these compounds affect the global cellular proteome remains unknown. Here, we use unbiased quantitative MS to identify the proteomic changes elicited by the VHL inhibitor compared with hypoxia or the broad-spectrum prolyl-hydroxylase domain enzyme inhibitor IOX2. Our results demonstrate that VHL inhibitors selectively activate the HIF response similar to the changes induced in hypoxia and IOX2 treatment. Interestingly, VHL inhibitors were found to specifically upregulate VHL itself. Our analysis revealed that this occurs via protein stabilization of VHL isoforms and not via changes in transcript levels. Increased VHL levels upon VH298 treatment resulted in turn in reduced levels of HIF-1α protein. This work demonstrates the specificity of VHL inhibitors and reveals different antagonistic effects upon their acute versus prolonged treatment in cells. These findings suggest that therapeutic use of VHL inhibitors may not produce overt side effects from HIF stabilization as previously thought.

This work was supported by the Wellcome Trust through a PhD Studentship to J. F. (102398), the European Research Council through a Starting Grant to A. C. (ERC-2012-StG-311460 DrugE3CRLs), and Cancer Research UK through a Senior Fellowship to S. R. (C99667/A12918).

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Abstract 2741

Protein design using deep learning

David Baker, *University of Washington*

Proteins mediate the critical processes of life and beautifully solve the challenges faced during the evolution of modern organisms. Our goal is to design a new generation of proteins that address current-day problems not faced during evolution. In contrast to traditional protein engineering efforts, which have focused on modifying naturally occurring proteins, we design new proteins from scratch to optimally solve the problem at hand. We now use two approaches. First, guided by Anfinsen's principle that proteins fold to their global free energy minimum, we use the physically based Rosetta method to compute sequences for which the desired target structure has the lowest energy. Second, we use deep learning methods to design sequences predicted to fold to the desired structures. In both cases, following the computation of amino acid sequences predicted to fold into proteins with new structures and functions, we produce synthetic genes encoding these sequences, and characterize them experimentally. In this talk, I will describe recent advances in protein design using both approaches.

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Topic Category Chromatin Structure, Remodeling and Gene Expression

Abstract 159

Functional partitioning of transcriptional regulators by patterned charge blocks

Heankel Cantu Oliveros, *University of Texas Southwestern Medical Center*

Reshma Veettil, Prashant Pradhan, Christy Fornero, Nancy De la Cruz, Keiichi Ito, Mikayla Eppert, Robert Roeder, Benjamin Sabari

Cellular components are selectively compartmentalized into different condensates often mediated by protein disorder, yet we know little about how this specificity is achieved. Here we show that condensates composed of the intrinsically disordered region (IDR) of MED1 selectively partition RNA Pol II together with its positive allosteric regulators while excluding negative regulators. This selective compartmentalization is sufficient to activate transcription and is required for gene activation during a cell state transition. The IDRs of partitioned proteins are necessary and sufficient for selective compartmentalization and require alternating blocks of charged amino acids. Disrupting this charge pattern prevents partitioning whereas adding the pattern to either natural or synthetic proteins promotes partitioning with functional consequences for gene activation. These findings demonstrate that proteins can be selectively compartmentalized by feature pattern matching within disordered regions.

This work was supported by Cancer Prevent and Research Institute of Texas (CPRIT) grant RR190090 to B.R.S and National Institutes of Health (NIH) grants GM147583 to B.R.S. and DK071900 and CA234575 to R.G.R. K.I. was supported by National Cancer Institute T32 grant CA009673 and by a Japan Society for the Promotion of Science postdoctoral fellowship for research abroad.

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Abstract 164

Enhancer regulation by H3K4me1 methyltransferases

Kai Ge, *NIDDK, NIH*

Enhancers control cell type-specific gene expression and are marked by H3K4me1. Active enhancers are further marked by H3K27ac. We identified MLL3/MLL4 (KMT2C/KMT2D) as major H3K4me1 methyltransferases and CBP/p300 as the H3K27 acetyltransferases in mammalian cells (EMBO J 2010; eLife 2013). During cell differentiation, MLL3 /MLL4 co-localize with lineage-determining transcription factors (LDTFs), CBP/p300, Brd4 and MED1 on active enhancers. MLL3 /MLL4 are required for enhancer activation, cell type-specific gene expression, and cell differentiation (eLife 2013; PNAS 2016; Nat Comm 2017). MLL3/MLL4 control cell differentiation by orchestrating CBP/p300-mediated enhancer activation (NAR 2017). Ectopic expression of H3.3K4M, an inhibitor of H3K4 methylation, or deletion of the SET domain, destabilizes MLL3/MLL4 proteins and prevents enhancer activation in cell differentiation and development (NAR 2019). We show an interdependent relationship between MLL3/MLL4 and the canonical SWI/SNF complex BAF in promoting cell type-specific enhancer activation by LDTFs, which rectifies seemingly conflicting results from previous studies (Nat Comm 2021). Interestingly, while the MED1 subunit of the Mediator coactivator complex marks active enhancers, it plays a cell- and gene-specific regulatory role and is not generally required for transcription (Genes Dev 2021). Our previous findings suggest that MLL4 regulates enhancer activation independent of H3K4me1 (PNAS 2016). To investigate the roles of MLL3/MLL4 enzymatic activities and H3K4me1 in enhancer activation, cell differentiation and development, we generated enzyme dead MLL3/MLL4 knockin mice and embryonic stem cells (ESCs). We found that constitutive elimination of both MLL3 and MLL4 enzymatic activities prevents initiation of gastrulation and leads to early embryonic lethality in mice. However, selective elimination of MLL3/MLL4 enzymatic activities in embryonic, but not extraembryonic, lineages leaves gastrulation largely intact. Consistently, embryonic stem cells (ESCs) lacking MLL3/MLL4 enzymatic activities can differentiate towards the three embryonic germ layers but show aberrant differentiation to extraembryonic endoderm and trophectoderm. The failure in extraembryonic endoderm differentiation can be attributed to markedly reduced enhancer-binding of the lineage-determining transcription factor GATA6. Furthermore, we show that MLL3/MLL4-catalyzed H3K4me1 is largely dispensable for enhancer activation during ESC differentiation. Together, our findings suggest a lineage-selective, but enhancer activation-independent, role of MLL3/MLL4 methyltransferase activities in early embryonic development and ESC differentiation (bioRxiv 2020).

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Abstract 1189

Transgenerational epigenetic inheritance of obesogenic traits in a *C. elegans* model

Abigale Wee, *The Nueva School*

Alexander Cocquyt, Giulia Kossev, Bo Lemkin, Rowan Tarpenning

C. elegans have been widely used as an obesity model, where the width of the organism is commonly used as a proxy for fat content, quantified by the width-to-length ratio. The wide range of experimental conditions—from varying glucose concentrations to diverse administration methods—complicates the comparison of width-length length ratios from one study to another that used slightly different experimental conditions. There is currently no way to compare the results obtained from the different methods and concentrations used. The project's first aim is to create a dataset containing width-length ratio data from worms raised on 0 mM, 50 mM, 100 mM, 150 mM, and 200 mM concentrations of glucose administered through seeding in NGM, a solution of the target concentrations pipetted on the surface of the NGM, and both methods to serve as a standard benchmark for the comparison and cross-examination of width-length ratios across studies. Here, we demonstrate a linear, direct, and dose-dependent relationship between higher glucose exposure and width-to-length ratios. Furthermore, the study of transgenerational epigenetic inheritance (TEI) of obesogenic information in *C. elegans* has previously been primarily concerned with physical phenotypic traits rather than behavioral ones, such as chemotaxis behavior to glucose. Thus, the project's second aim is to show that upon the exposure of a generation of *C. elegans* to a high glucose diet (HGD), their progeny will exhibit higher chemotaxis indices to glucose and higher fat content (larger width-length ratio) as compared to a control group of *C. elegans*. We prove that the higher chemotaxis indices and width-length ratios observed in the progeny of a generation of *C. elegans* to an HGD occur through TEI via knockdown of wdr-5.1 and mes-4 (genes that regulate TEI of chemotaxis behavior and fat accumulation). This research can potentially expand the use of *C. elegans* as a model obesity organism in the study of epigenetic inheritance and validate the use of a highly-accessible assay for fat content in *C. elegans*.

This research was funded by The Nueva School. The Shen Lab at Stanford University provided access to equipment, including a fluorescence microscope. N2 *C. elegans* were provided by the Caenorhabditis Genetics Center at the University of Minnesota.

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Abstract 1202

Whamy is Involved with Actinmyosin Ring Constriction During Drosophila Cellularization

Mirika Jambudi, *The Pingry School*

In *Drosophila melanogaster*, Wiskott-Aldrich syndrome (WASP) family proteins play a critical role in many cellular processes involving reorganization of the F-actin cytoskeleton. Subfamily members Scar, Wash, WASp, and Whamy, have been implicated as essential during early Drosophila development. While studies have investigated the role of WASp and other isolated subfamily WAS proteins (Washout and Scar), the function of Whamy remains unclear. However, its association with actin, membranes, and microfilaments during early embryogenesis suggests a role in the microfilament cytoskeleton. Since it has been established that WASP family proteins function as a connector between the cell membrane and Arp2/3 complexes to polymerize F-actin during cytoskeleton development, this study focused on elucidating the role of Whamy during cellularization and microfilament ring constriction by analyzing the mutant phenotype. Wild-type (WT) OreR embryos and mutant Whamy embryos were collected and stained with Neurotactin and Zipper antibodies to visualize cell membranes and myosin in the embryos during cellularization. I then performed quantitative analysis of the microfilament rings using ImageJ and QuPath software. Quantification showed a two-fold increase in the number of microfilament rings in the mutant Whamy compared to WT, which suggests that the mutant protein induces cells to go through an additional cell cycle. Imaging of the Whamy microfilament rings showed that the rings undergo constriction at a much earlier stage than WT microfilament rings. These abnormalities in microfilament ring development during cellularization suggest that Whamy is necessary for normal development of the microfilament rings and that mutations in this protein may contribute to defects in cytoskeletal development.

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Abstract 1218

Genetic determinants of ammonium excretion in nifL mutants of *Azotobacter vinelandii*

Florence Mus, *Washington State University*

Nathaniel Boyer, Devanshi Khokhani, April MacIntyre, Ray Dixon, Jean-Michel Ané, John Peters

Since the Green Revolution, N fertilizers provided by the Haber-Bosch process have become an essential part of modern agriculture, sustaining crop yields and replacing N removed from the system at harvest. However, with the increasing global population, problems caused by unintended N leaching and the production of greenhouse gases have led to a global "nitrogen problem" More sustainable ways of managing the N cycle in soil and utilizing biological nitrogen fixation (BNF) are now imperative. Interference with the mechanisms by which ammonium inhibits either nitrogenase synthesis or activity has been considered strategies to increase the amount of fixed nitrogen transferred from bacteria to the plant partners. The manipulation of soil diazotrophs can potentially provide a means to reduce the use of synthetic nitrogen fertilizers, thus providing a solution to the nitrogen problem. The ubiquitous diazotrophic soil bacterium *Azotobacter vinelandii* has been extensively studied as a model organism for BNF. In *A. vinelandii*, BNF is regulated by the NifL-NifA two-component system, where NifL acts as an anti-activator that tightly controls the activity of the nitrogen fixation-specific transcriptional activator NifA in response to redox, nitrogen, and carbon status. While several studies reported that mutations in *A. vinelandii* nifL resulted in the deregulation of nitrogenase expression and the release of large quantities of ammonium, knowledge about the specific determinants for this ammonium-excreting phenotype is lacking. In this work, we report that only specific disruptions of nifL lead to large quantities of ammonium accumulated in liquid culture (~12 mM). The ammonium excretion phenotype is associated solely with deletions of NifL domains combined with the insertion of a promoter sequence in the orientation opposite that of nifLAtranscription. We further demonstrated that the strength of the inserted promoter could influence the amounts of ammonium excreted by affecting rnf1 gene expression as an additional requirement for ammonium excretion. These ammonium-excreting nifL mutants significantly stimulate the transfer of fixed nitrogen to rice. In addition, we are investigating the structure of NifL and the conformational dynamics involved in NifL-specific regulation of NifA activity in response to oxygen, energy, and nitrogen status. In lieu of an experimentally determined structure, we have generated a structural model of the NifL dimer. Complementary data collected from small angle x-ray scattering and mass spectrometry coupled surface labeling of NifL samples poised in specific conformations served as restraints to refine our initial model, generating conformation-specific NifL models. This work provides important new insights into how NifL responds to

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oxygen, energy, and nitrogen status to regulate NifA-directed transcription.

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Abstract 1265

Examining the Requirement of Coactivators in Enhancer Regulation in mouse embryonic stem cells (ESCs)

Abrahan Vasquez, *Claremont McKenna College*

Ryan Boileau, Robert Blelloch

The epithelial to mesenchymal transition (EMT) is relevant to cancer studies since it is responsible for cell migration, an essential component of metastasis. In examining the epigenetics of cell transitions such as the EMT, it is important to understand the interactions between different non-coding regions of DNA due to their gene regulatory effects. Specifically, investigating enhancer activation allows for an understanding of three-dimensional enhancer-promoter interactions. The transcription factor Grainyhead-like 2 (GRHL2) can suppress the EMT in certain cell types and is involved in bringing together enhancers and promoters through recruitment of the cofactor protein cohesin. Here, we use a GRHL2-Estrogen fusion construct that allows for a reductionist model of the transition from naive embryonic stem cells (ESCs) to formative epiblast-like cells (EpiLCs), a transition relevant to the EMT since it is an *in vitro* substitute for early cell fate differentiation, and it features GRHL2 enhancer-switching. This reductionist system has allowed for an investigation of the transcriptional requirement of the coactivator protein subunits, RAD21, BRG1, and p300/CBP. Preliminary data from the Blelloch lab suggests that some of these coactivators, namely RAD21 are not required for transcription, and a time-course degradation of these coactivators and qPCR RNA level analysis of Cldn6 and Wnt7b, two genes relevant to tumorigenesis, further confirms the dispensability of RAD21 but suggests further optimizing of the model system for the other coactivators. With the requirement of RAD21 now in question, future research will center on optimization of the model and on resolving the order of coactivator activity.

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Abstract 1340

Nuclear biomolecular condensates regulate transcriptional responses during stress

Sethu Pitchiaya, *University of Michigan-Ann Arbor*

Joel Berends, Vijaya Dommeti, Jeffrey Dudley, Giovana Veronezi, Srinivas Ramachandran, Srinivas Ramachandran

When mammalian cells are stressed, they invoke evolutionarily conserved measures that culminate in the global suppression of RNA and protein synthesis, selective expression of stress response genes and accumulation of biomolecules within subcellular condensates. Aberrant stress response, especially defects in the formation or dissolution of condensates, is linked to a variety of degenerative and neoplastic pathologies. However, the mechanism by which cells achieve transcriptional selectivity during stress, and whether stress-induced biomolecular condensates are involved in this process are largely unknown. We recently discovered that a significant fraction of the multimeric proteome rapidly and reversibly assembles within distinct subcellular condensates in response to hyperosmotic stress, with widespread impact on RNA metabolism. Here, we find that trimeric heat shock factors (HSFs), transcription factors that help counteract proteotoxicity, not only undergo hyperosmotic phase separation (HOPS) but also form nano-to-mesoscale nuclear condensates in response to a variety of other stresses (e.g. heat shock, oxidative stress, hypoxia and proteotoxic drugs). While HSF condensate formation is generic to all stresses, the physicochemical properties of HSF condensates vary by stress, and the process is independent of the integrated stress response. Moreover, metabolic RNA labeling followed by click chemistry assisted fluorophore conjugation, coupled with multi-color super-resolution microscopy, showed that HSF condensates adjacently localize to nascent RNA hubs during heat shock but not during other stresses, suggestive of stress-type-specific transcriptional activity and formation of spatial regulons. Concordantly, epigenomic analysis by Cleavage Under Targets and Tagmentation (CUT&Tag) combined with nascent RNA sequencing by bromouridine-mediated metabolic RNA labeling (BrUseq) revealed that HSF1 genomic occupancy varies by stress to drive stress-type dependent transcriptional programs. Our data shows that HSF condensates have context-specific functions that distinctly affect cell fate decisions during stress and possibly explains why drugs targeting the proteostasis axis have different therapeutic outcomes in distinct diseases. Overall, our work suggests that the formation of nuclear condensates is a central, possibly therapeutically tractable, stress response mechanism that regulates transcription and occurs in parallel to formation of cytoplasmic stress granules.

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Abstract 1383

Novel Transcription Factor Linking Pulcherrimin to Cell Death

Gillian McClennen, *Northeastern University*

Leticia Lima Angelini, Yunrong Chai

Pulcherrimin is a reddish, iron-chelating pigment that is secreted by certain yeasts and bacteria as a way to compete with other microbes for environmental iron. The newly identified bacteriostatic effects of pulcherrimin and its known iron-binding capacity renders it an interesting tool to study microbial interactions and a potential bioproduct with applications in food and agricultural industries. In this project, we used the pulcherrimin-producing bacterium *Bacillus subtilis* to investigate the role of YhjH, a novel MarR-type transcription factor, in the genetic regulation of pulcherrimin biosynthesis. Molecular biology techniques were used to create *B. subtilis* strains expressing different levels of yhjH, and an interesting phenotype was observed; deletion of yhjH significantly reduces pigment production, while overexpression of yhjH leads to cell death. To elucidate these phenomena, interactions between yhjH and other genes in the pulcherrimin production pathway (yvmB and yvmC) were explored through further genetic manipulation and biochemical methods. The DNA binding site of YhjH was determined through promoter walk and site-directed mutagenesis. Additionally, a genome-wide transcriptome profiling was performed using RNA-Seq to determine the regulon of YhjH. In summary, YhjH is a newly characterized transcription factor shown to be involved in the regulation of pulcherrimin production in *B. subtilis*, and its overproduction triggers programmed cell death via a yet unknown mechanism. Through this work, we propose an updated model of pulcherrimin regulation and environmental control.

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Abstract 1394

Advanced computational modeling of molecular machines involved in transcription initiation and DNA repair

Ivaylo Ivanov, *Georgia State University*

Chunli Yan, Jina Yu, Susan Tsutakawa, Chi-Lin Tsai, John Tainer

Transcription pre-initiation complexes (PIC) are vital assemblies whose function underlies protein gene expression. Cryo-EM advances have begun to uncover their structural organization. Yet, functional analyses are hindered by incompletely modeled regions. This talk will describe our recent studies aimed at structural and mechanistic characterization of pre-initiation complexes. Recently, we integrated available cryo-EM data to build a human PIC structural model, suitably complete to allow computational modeling of its functional dynamics. This enabled large-scale dynamics simulations on the Summit machine at the Oak Ridge Leadership Computing Facility that revealed the assembly's global motions, defined PIC partitioning into dynamic communities and delineated how its interwoven structural modules function together to remodel DNA. We identified key TFIIE–p62 interactions linking core-PIC to transcription factor IIH (TFIIH) – a general transcription factor with dual functions in transcription and in nucleotide excision repair of DNA damage. We found that the p62 subunit rigging interlaces the p34, p44 and XPD subunits of TFIIH while capping the DNA- and ATP-binding sites of XPD. The identified global motions of the assembly allow PIC to kink and lock substrate DNA within the Pol II cleft and, in turn, this underwound DNA facilitates promoter opening. Strikingly, a mapping of TFIIH disease mutations onto the newly discovered dynamic communities showed that mutations were clustered at critical junctures in the TFIIH dynamic network. These findings shed light on the PIC/TFIIH molecular mechanisms and the etiology of three genetic disorders – xeroderma pigmentosum, trichothiodystrophy and Cockayne syndrome – associated with cancer, aging, and developmental defects.

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Abstract 1418

Testing and Optimization of piggyBac Using Transposase mRNA and Transposon DNA Towards Gene Therapy for Kidney Disease

Krystal Rivera, *Vanderbilt University*

Anna Menshikh, Wentian Luo, Matthew Wilson

The piggyBac transposon system is a non-viral gene delivery system that efficiently integrates transgene DNA into the cellular genome. The system works by a transposase enzyme cutting transposon DNA, which contains terminal inverted repeats (TIRs) at both ends of the transposon flanking the transgene cargo, and then pasting the transposon into the genome. Transposons are useful integrating vectors for a variety of applications from making stable cell lines to gene therapy. Previous research has used plasmid DNA for expression of piggyBac for genetic modification for kidney and other diseases; however, plasmid DNA is not stable and does not enable long-lasting expression of the transgene and thus, integrating stable and long-lasting transgene expression remains a major challenge for gene therapy. Here we show that delivery of the piggyBac transposase messenger RNA achieves efficient transposition and stable genomic integration in human HT-1080 cells. Ultimately, we analyzed the efficiency and stability by colony count and performed excision protocol to determine the activity of the transposon system. Using piggyBac transposase mRNA in human cells may prove improve stability and can help establish new approaches for kidney-targeted gene transfer paving the way for new therapies for kidney disease.

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Abstract 1428

Dysregulation of Epigenetic Condensates in Human Disease

Hao Jiang, *University of Virginia*

Wei Li, Bi Shi, Yansu Song, Jing Hu

We study how the liquid-liquid phase separation mechanism spatiotemporally regulates gene expression in development and disease including cancer. We show that AKAP95, a nuclear protein that integrates transcription and RNA splice regulation, plays an important role in sustaining tumorigenesis. AKAP95 forms phase-separated liquid condensates *in vitro* and in cells. Through mutagenesis and replacement of the disordered region, we demonstrate a crucial functional role of AKAP95 phase separation in its activities in regulating splicing and tumorigenesis. Moreover, a mutation that hardens the condensates also impairs its activity in regulating splicing and tumorigenesis. Therefore, the ability of forming condensates with proper fluidity and dynamicity is crucial for the biological activity of AKAP95. These findings also suggest the unconventional possibilities to treat cancer by either disrupting or hardening the cancer-promoting condensates. UTX/KDM6A is a histone H3K27 demethylase frequently mutated in cancer, but its demethylase activity is often dispensable for tumor suppression, leaving the underlying molecular mechanisms elusive. We show that a core intrinsically disordered region of UTX forms phase-separated liquid condensates, and loss of this region by the most frequent cancer mutation is mainly responsible for abolishing tumor suppression. By mutagenesis and replacement of the disordered region, we demonstrate a critical role of UTX condensation in tumor suppression. By genomic editing, we show that condensation of the endogenous UTX is important for embryonic stem cell differentiation. As shown in *in vitro* reconstitution and engineered cell systems, UTX recruits MLL4 and p300 into co-condensates *in vitro* and on chromatin, and enriches the H3K4 methylation activity of MLL4. Integrated genomic analyses indicate that UTX condensates regulate multi-level chromatin activity, including high-order chromatin interactions, to orchestrate a transcriptional program important for tumor suppression. We also show that UTY, the Y chromosome homolog of UTX, has weaker tumor-suppressive activity because it forms more solid-like and less dynamic condensates. Moreover, certain cancer-associated UTX mutations alter the condensate properties. These results demonstrate a crucial biological function of epigenetic condensates in tumor regulation, and that natural variations in condensates properties may contribute to the widely observed sex bias in cancer. We are also studying how dysregulation of phase separation by mutations in UTX and other epigenetic proteins cause developmental disorder and cancer. Our data suggest a complex interplay of the structured and unstructured protein regions in regulating phase separation, and show other functions of disordered regions in modulating (not merely promoting) phase separation for important biological processes.

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Abstract 1441

Viral chromatin integrity enables transcription of the Hepatitis B Virus oncogene HBx

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Andres Mansisidor, Yaron Bram, Abigail Lemmon, Viviana Risca, Robert Schwartz, Yeal David

Chronic Hepatitis B Virus (HBV) infection is a global public health threat responsible for almost half of all cases of hepatocellular carcinoma. The key replicative intermediate and transcriptional template of HBV is a viral minichromosome composed of covalently closed circular (ccc)DNA. While existing antiviral treatments prevent viral proliferation, an inability to target cccDNA allows the minichromosome to persist and support chronic infection. Host histones populate cccDNA, and studies have shown they are heavily enriched with activating post-translational modifications (PTMs) compared to bulk host chromatin. Moreover, the primary host effector protein encoded by HBV, Hepatitis B protein X (HBx), has been shown degrade the Smc5/6 complex, which otherwise silences cccDNA transcription. These data lead to a seemingly paradoxical relationship in which HBx is required for viral transcription, but cccDNA must be transcribed to generate HBx in newly infected cells. To understand this paradoxical relationship, I developed a platform to reconstitute the viral minichromosome for biophysical and biochemical interrogations of the system. Unexpectedly, *in vitro* transcription of empty or chromatinized cccDNA revealed a dependence on chromatin integrity for the generation of viral RNA encoding HBx, but not other viral transcripts. Furthermore, pharmacological treatment of mammalian cells with nucleosome-destabilizing small molecules dramatically reduced the transcriptional capacity of cccDNA but not host genes. Together, these data suggest a model whereby rapid histone incorporation around the HBx transcription start site permits infection establishment prior to Smc5/6-mediated viral silencing. These results thus pose an exciting opportunity to develop novel therapeutics targeting the chromatin structure and organization of cccDNA in chronic HBV infection.

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Abstract 1486

Spontaneous Histone Exchange Between Nucleosomes

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Study Objective: Understanding the role of DNA accessibility in spontaneous histone exchange between nucleosomes, and in kinetics of histone exchange rates. Abstract In eukaryotes, the nucleosome is the primary gene-packing unit structure. The octameric histone protein core of nucleosomes, which consists of two H2A-H2B dimers and one (H3-H4)2 tetramer, is wrapped by 147 bp of DNA. The dynamic interactions between DNA and histones regulate DNA access while the strong DNA-histone interactions impose physical barriers to DNA binding and processing. According to the dynamics, nucleosomes at least momentarily break down DNA-histone interactions. On a long-term basis, this kinetic instability could result in spontaneous nucleosome disassembly or histone exchange between nucleosomes upon their contact. Nucleosome-nucleosome collisions and subsequent histone exchange, in which nucleosomes serve as their own chaperone, would be a more probable pathway at high nucleosome concentrations. Although it has never been reported, this spontaneous histone exchange between nucleosomes would act as a mechanism for maintaining the overall stability of chromatin. We used three-color single-molecule FRET (smFRET) to show that histone H2A-H2B dimers spontaneously swap across nucleosomes and that the time scale is on a few tens of seconds at a physiological nucleosome concentration. We tested the effects of various chromatin modifications, ionic conditions, and temperature on the kinetics of histone exchange such as CpG methylation (suppressed exchange), histone H3 K56 acetylation (facilitated exchange), higher salt conditions (facilitated exchange), and higher temperature (no apparent change in the kinetics). These findings validate spontaneous histone diffusion in a confined chromatin setting and support histone exchange by transitory and recurrent partial disintegration of the nucleosome, regulating the local concentrations of post-translational histone modifications and histone variations.

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Abstract 1501

CBD Treatment Improves Fatal Inflammatory Response Exhibited in SEB-Induced ARDS

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The novel SARS-CoV-2 virus known to cause the COVID-19 outbreak has resulted in a global healthcare crisis that has persisted the past 3 years. Thus, understanding the mechanisms underlying this disease are vital at this time. While there are issues of research infrastructure to handle the virus and because of the refractoriness of rodents to this disease, the availability of these tools is still limited. The cytokine storm and fatality presented in patients with severe COVID-19 can be mimicked with Staphylococcal enterotoxin B (SEB)-induced Acute Respiratory Distress Syndrome (ARDS). Within ~7 days, the survival rate drops to 0% for C3H/HeJ mice exposed to a dual dose of SEB. In this study, we administered cannabidiol (CBD) intraperitoneally for 3 days pre- and post-SEB dosing and found that the clinical outcomes improved significantly. Initial evaluation of scRNASeq data from lungs comparing naïve to SEB-induced ARDS mice illustrated an increase in infiltrating immune cells, and a loss in pulmonary epithelial cells in the latter group. When evaluating the effect of CBD treatment on SEB-induced ARDS, we were able to demonstrate that CBD reduced the macrophage population. To characterize the mechanism by which CBD treatment ameliorated the inflammatory response, we found that CBD treated mice had significant reduction in infiltrating immune cells and alveolar thickening. This same histology and infiltration is presented in ARDS. MicroRNA expression analysis showed a significant increase in the expression mmu-miR-298-5p and mmu-miR-566 with CBD treatment. Ingenuity Pathway Analysis (IPA) indicated that the dysregulated miRNAs were also implicated in pathways associated with macrophage activation, respiratory disease and inflammation, interferon stimulated genes, as well as genes which have been upregulated in the disease state of this model. These targets include but are not limited to Cebpb, Efhd2, Stat3, Socs3, Cxcl5, Gbp2, and Birc3. This finding offers insights for the development of preventive and therapeutic strategies in the treatment of ARDS, including that induced in COVID-19.

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Abstract 1534

Hormone-induced enhancer assembly requires an optimal level of hormone receptor multivalent interactions

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Transcription factors (TFs) activate enhancers to drive cell-specific gene programs in response to signals, but our understanding of enhancer assembly during signaling events is incomplete. Here, we show that Androgen Receptor (AR), a steroid hormone-regulated transcription factor, forms condensates through multivalent interactions in response to androgen signaling to orchestrate enhancer assembly. We demonstrate that the intrinsically disordered N-terminal domain (NTD) of AR drives 1,6-Hexanediol-sensitive condensate formation and that NTD deletion or aromatic residue mutation reduces AR self-association and abolishes AR transcriptional activity. AR NTD can be substituted by intrinsically disordered regions (IDRs) from selective proteins for AR condensation capacity and transactivation function. Surprisingly, strengthened AR condensation capacity caused by extending the polyQ tract within AR NTD also leads to impaired transcriptional activity without affecting AR binding on enhancers. Furthermore, either NTD deletion or polyQ extension reduces heterotypic multivalent interactions between AR and other enhancer components. These results suggest the importance of an optimal level of AR condensation in mediating AR-AR homotypic and AR-cofactor heterotypic interactions to regulate enhancer assembly in response to signals. Our study supports the notion that alteration of the fine-tuned multivalent IDR-IDR interactions might underlie AR-related human pathologies, thereby providing novel molecular insights for potential therapeutic strategies to treat prostate cancer and other AR-involved diseases by targeting AR multivalent interactions.

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Abstract 1558

Decoding the regulation of genome-wide accessible chromatin and gene expression in the polyploid Camelina

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Camelina sativa is an important emerging allohexaploid oilseed crop. Subgenome dominance and homoeolog expression bias are common phenomena among allopolyploid species, including Camelina, resulting from broad genetic and epigenetic changes that arise due to "genome shock" after polyploidization. However, little is known about the mechanisms underlying the alteration in chromatin and gene expression dynamics after the allopolyploidization process. In this study, by using Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq), we are identifying the accessible chromatin regions (ACRs) and their association with the control of gene expression during seed development in Camelina. We performed ATAC-seq in five embryo developmental stages (15, 19, 23, 27, and 30 days post anthesis; DPA). We identified ~70 000 high-confidence ACRs, which were widely distributed throughout the genome, with the highest enrichment at the promoter region and mid-enrichment at intergenic and transcription end sites. Analysis of chromatin accessibility dynamics provides evidence that regulatory regions of genes related to fatty acid synthesis and lipid-related metabolic pathways were more accessible at 19–23 DPA and slightly less accessible at 15, 27, and 30 DPA. Similarly, the chromatin region of genes related to seed embryo development was more accessible at 15 DPA, whereas dormancy-related genes were gradually more accessible after 23 DPA. These results are currently being integrated with DNA affinity purification high-throughput sequencing information recently acquired for a set of ~15 candidate fatty acid regulators. All these results will be integrated into CamRegBase (https://camregbase.org/). The results shown in this study highlight valuable resources regarding the genome-wide landscape of binding sites for transcription factor and chromatin regulators during seed development and contribute to understanding their evolution for improvements of potential agronomic traits.

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Abstract 1564

Design rules for multiple bacterial CRISPRa systems enable synergistic gene activation

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The development of effective CRISPR gene activation (CRISPRa) tools has the potential to dramatically accelerate bacterial strain engineering. Unlike in eukaryotic CRISPRa systems, effective transcriptional activation with bacterial CRISPRa requires precise placement of transcriptional activators relative to a targeted promoter. Different transcriptional activators have distinct mechanisms for recruiting RNA polymerase (RNAP), which impacts the preferred target site positions for effective CRISPRa. Here, we employed a set of engineered, synthetic promoters to investigate distance rules with different bacterial activator proteins. Across the set of CRISPRa effector proteins tested, we found that optimal activator placement relative to the transcription start site (TSS) could vary up to 200 base pairs. Using these guidelines, we tested multiple activators in combination and found that CRISPRa mediated by SoxS and PspF effectors could synergistically activate gene expression. Many other combinations of effectors were antagonistic. This work demonstrates that synergistic CRISPR transcriptional activation can be achieved in bacteria through a systematic investigation of design rules.

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Abstract 1590

Optimization of CHIP seq protocol to explore SUMOylated DNA binding proteins in human cardiomyocytes

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Chromatin immunoprecipitation sequencing (CHIP seq) is a prevailing method to identify histone modifications and binding sites of transcription factors (TF). In addition to various cis and trans regulatory factors, the binding of TF to chromatin is dependent on their post translational modifications (PTM). SUMOylation is a well-known PTM, vital in deciding the fate of various regulatory proteins including transcription factors. Mammals express three major SUMO paralogues which regulate the expression of genes and protein fate in cells, development of organs, their homeostatic functioning and eventually the physiology of host. Here we present a detailed protocol for CHIP seq to identify SUMOylation of transcription factors (TF) and other DNA binding proteins (DBP) in human cardiomyocytes (AC16). We optimized the procedure step-by-step to enhance the yield of immunoprecipitated chromatin. The cells were fixed with paraformaldehyde without methanol for 10 min followed by chromatin fragmentation by sonication. The size of chromatin fragments was visualized on agarose gel after phenol-chloroform extraction. The chromatin immunoprecipitation was done with SUMO1, SUMO2/3, RNA polymerase and IgG antibodies. The quality of CHIP assay was assessed by qubit and qRT-PCR. The immunoprecipitated chromatin (0.5 to 2 ng) was used for library preparation and further amplification. The bioanalyzer profiling of library was done followed by sequencing. The amount of chromatin obtained using this protocol generates a good library for sequencing, in approximately 7 days, by researchers experienced in molecular biology laboratory work. The complete method was implemented manually which detected thousands of transcripts with binding sites for SUMOylated TF and other DBP in the human cardiomyocytes. The Mean read quality (Phred score) lies in between 34 to 36 with a mean of 151 base pairs. Sequencing data analysis including alignment, peak calling and identification of TF motif requires a knowledge of Linux platform and understanding for software's such as Bowtie, MACS2 and HOMER. The analysis revealed several putative targets that can be used to identify the effects of PTM in various pathological and stress conditions.

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ASBMB

Abstract 1602

The Effects of Histone H2B ubiquitylations and H3K79me3 on Transcription Elongation

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Bhaswati Sengupta, Wladyslaw Krajewski, Tae-Hee Lee

The nucleosome is the basic packing structure of DNA in the eukaryotic genome, comprising an octameric histone protein core and ~147 bp DNA. Histone post-translational modifications (PTMs) can mediate gene regulation by altering the global and local stability of the nucleosome. In this study, we employed single-molecule FRET to investigate the effects of semi-synthetically introduced histone H2B ubiquitylations at K34 (H2BK34ub) and K120 (H2BK120ub), and H3K79 trimethylation (H3K79me3) on the kinetics of transcription elongation by RNA Polymerase II (Pol II). We investigated the non-modified nucleosome and four modified nucleosomes, each of which is modified with H3K79me3, H2BK34 ubiquitylation (H2BK34ub), H2BK120 ubiquitylation (H2BK120ub), or H3K79me3/H2BK120ub. A nucleosome-containing transcription template was constructed by ligating DNA fragments followed by nucleosome assembly. DNA was labeled with a FRET pair Cy3 and Atto647N that reports DNA unwrapping and rewrapping during and after transcription elongation. The overall efficiency of transcription is controlled by Pol II pauses, which can last anywhere from a few seconds to minutes at various sites within the nucleosome. By monitoring the changes in Cy3 and Atto647N relative intensities in real-time, we found that H2B ubiquitylations expedite transcription near the nucleosome entry while H3K79me3 slightly alleviates the pause near the dyad and increases the elongation rate. We also observed increased DNA rewrapping after Pol II transcription in H2BK34ub nucleosomes. These findings suggest that H3K79me3 promotes Pol II progression by disrupting the local nucleosome structure, while H2B ubiquitylations promote transcription elongation and aid in maintaining the chromatin structure by inducing and stabilizing nucleosome intermediates. Our findings outline the processes by which these alterations, when joined by a network of regulatory proteins, enable transcription in two distinct locations in the nucleosome and support the preservation of chromatin structure while transcription is active.

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Abstract 1622

Combinatorial effects of the ACT-like domain and small molecule on the regulatory activity of the maize bHLH transcription factor R1

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In the maize aleurone, the basic helix-loop-helix (bHLH) transcription factor R interacts with the R2R3-MYB regulator C1 to activate the anthocyanin biosynthesis pathway. However, it is unclear how the coordinate regulation of all the pathway genes is accomplished, without any obvious conservation of the respective regulatory regions. Our previous studies showed that the monomer/dimer configuration of an ACT-like domain at the C-terminus of R affects the DNA-binding activity of the R bHLH motif. We proposed a model in which when the ACT-like domain forms a dimer, the bHLH is monomeric and R is tethered to DNA indirectly, through the interaction with C1. When the dimerization of the ACT-like is impaired, then the bHLH motif dimerizes and recognizes a canonical G-box (CACGTG). To further elucidate the mechanisms by which the ACT-like domain affects DNA-binding by the bHLH motif, we examined the *in vitro* DNA binding capacity and kinetics of the bHLH motif on the promoter region of anthocyanin biosynthetic gene A1 and canonical G-box in the absence/presence of the ACT-like domain. Using amplified luminescent proximity homogeneous assay (ALPHA), we identified non-canonical DNA-binding sites of the bHLH motif in the A1 promoter and demonstrated that, in the presence of the ACT-like domain, the binding of bHLH to the A1 promoter or canonical G-box shows similar affinity (KD) but different kinetics (Kon and Koff rates). Preliminary results indicated small molecules, including flavonoid pathway intermediates, contribute to the formation of different transcriptional complexes through interactions with the ACT-like domain. Results will be discussed in the context of what this means for the regulation of the anthocyanin pathway, and for how ACT-like domains present in ~30% of the plant bHLH transcription factors influence gene regulation.

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Abstract 1642

Determining the contribution of histone variant H2A.Z to the promotion of the epigenetic mark H3K27me3 in *Arabidopsis thaliana*

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Histone variant proteins play a critical role in chromatin-based transcription, and their specific contributions to chromatin states and consequent gene expression are an area of intense investigation. The histone variant protein H2A.Z is widely conserved in animals, plants, and yeast and is thought to make chromatin more dynamic by altering nucleosome modification states and chromatin accessibility. We have proposed a model by which H2A.Z contributes to deposition of the repressive histone modification H3K27me3 in Arabidopsis. H3K27me3 silences genes by promoting a distinct chromatin environment, and the loss of H3K27me3 is associated with failed development and callus formation (undifferentiated growth) in plants. Our previously published data indicate that PIE1, a SWR1 remodeler, promotes both the histone variant H2A.Z and H3K27me3. Although these data raise the possibility that SWR1 promotes H3K27me3 in an H2A.Z-dependent fashion, it is also possible that SWR1 acts via another mechanism. To test the hypothesis that the loss of H2A.Z is sufficient to alter H3K27me3 levels, we examined H3K27me3-dependent traits in plants that lack two genes that encode H2A.Z, HTA9 and HTA11. We have specifically utilized hta9 hta11 plants that also lack the chromatin remodeler PKL because pkl plants exhibit an embryonic phenotype called pickle root that is strongly associated with reduced levels of H3K27me3. We conducted a pickle root penetrance analysis and determined that hta9 hta11 pkl plants exhibit a significant increase in pickle root penetrance compared to pkl plants. Using this phenotypic evidence as a proxy for relative levels of H3K27me3, these data are consistent with the possibility that the loss of H2A.Z is sufficient to decrease H3K27me3 levels in the absence of PKL. Phenotypic characterization of other H3K27me3-associated traits such as bolting time, flowering time, height, and rosette diameter is ongoing and will shed further insight on how reduction of H2A.Z affects these traits in the absence of PKL. We will also perform qRT-PCR to directly examine gene expression and chromatin immunoprecipitation to examine enrichment of H3K27me3 at H3K27me3-enriched loci in these lines. The combined results of these analyses will substantially clarify the role that H2A.Z plays in H3K27me3 homeostasis and potentially illuminate how H2A.Z contributes to developmental fluidity in plants.

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Abstract 1660

Effects of acetyllysine modification on histone H4 tail structure and dynamics

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Chromatin accessibility underlies fundamental cellular processes including proliferation and differentiation, DNA replication and damage repair, recombination, and transcription. The intrinsically disordered tail of histone H4 forms inter and intra-nucleosome contacts that are implicated in chromatin compaction, nucleosome accessibility, and recognition by chromatin associated proteins. Acetyllysine modification of the H4 tail can abrogate formation of higher order chromatin structures *in vitro* and is correlated *in vivo* with active DNA elements in open chromatin environments, yet the biophysical basis for these observations remains primarily theoretical. Beyond conspicuous charge neutralization, computational studies predict that acetyllysine modification alters ensemble behavior of the H4 tail. These predictions could provide a structural basis for the relationship between acetyllysine modification of the H4 tail and chromatin accessibility; however, they have not been interrogated experimentally. Here we leverage nuclear magnetic resonance spectroscopy alongside orthogonal biophysical methods to characterize changes to dynamics, compactness, and structural propensity in the histone H4 tail ensemble following acetyllysine modification. The results provide structural insight regarding how acetyllysine modification of the histone H4 tail may affect chromatin structure, nucleosome accessibility, and recognition by chromatin associated proteins with important implications for continued investigation into the role of acetyllysine modification as a prevalent regulator of DNA templated cellular processes.

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Abstract 1666

Deciphering the role of TGFβ in regulating transcriptome and DNA methylome during EMT to identify therapeutic targets for clear cell renal cell carcinoma

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Clear cell renal cell carcinoma (ccRCC) is the most common subtype of renal cell carcinoma (RCC) and accounts for majority of the deaths from renal cancer. The TGFβ signaling pathway is known for its pro-oncogenic effects in promoting cell survival, invasion, and immunosuppression. Although several studies have indicated that TGFβ is able to induce epithelial to mesenchymal transition (EMT) in kidney cell lines, the mechanism by which TGFβ alters the epigenome and transcriptome during EMT is not well-understood. Here, by characterizing temporal changes in gene expression and DNA methylation through RNA-seq analysis and Illumina 850k methylation profiling in a non-tumorigenic renal epithelial cell line, we found that TGFβ induces dramatic changes in the transcriptome and promotes global DNA hypermethylation. Those hypermethylated CpG sites are enriched in intergenic regions that likely regulate enhancer activity. Ingenuity pathway analysis shows that cytoskeleton remodeling, integrin signaling, EMT and metastasis are particularly enriched for genes that are consistently upregulated throughout 72 h TGFβ treatment. Future therapeutic methods targeting these oncogenic pathways, or their associated genes could be novel treatment options for ccRCC. In summary, our findings reveal that TGFβ significantly alters the transcriptome and DNA methylome, and further uncovers several pathways could be therapeutically targeted for treatment of ccRCC patients.

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Abstract 1691

Asp251 of the DEAF1 KDWK binding motif influences binding to flexibly spaced consensus sequences

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Introduction: Deformed epidermal autoregulatory factor 1 (DEAF1) is a transcription factor important in early embryonic and neural development. Pathogenic variants in the DEAF1 gene cause a spectrum of neurodevelopmental disorders including autism, intellectual disability, speech, and motor developmental delay - collectively referred to as DEAF1-associated neurodevelopmental disorders (DAND). DEAF1 binds flexibly spaced TTCG DNA half-sites through a conserved KDWK motif (aa250–253) within the DNA binding domain (DBD). Identified mutations at Lys250, Trp252, or Lys253 eliminate DNA binding and transcriptional activity, but the effects of Asp251 on DEAF1-DNA interactions are unclear. This study aims to further characterize the DEAF1 KDWK binding motif by assessing the effects of Asp251 amino-acid substitutions on DNA binding and transcriptional function.

Methods: Exome sequencing of an individual with autism and severe intellectual disability, identified a DEAF1 missense variant within the KDWK binding motif (p.Asp251Asn). DEAF1 plasmids expressing p.Asp251Asn, as well as 3 additional amino-acid substitutions (p.Asp251Ala, p.Asp251Glu, and p.Asp251Gln) were generated using site-directed mutagenesis. Dual luciferase transcription assays were performed to determine the effects of Asp251 mutations on DEAF1 transcriptional repression and activation activity. Electrophoresis mobility shift assays (EMSA) were conducted to assess the binding affinity of the Asp251 substitutions on 3 DEAF1 DNA ligands (flexibly spaced half-sites separated by 6, 9, or 11 nucleotides).

Results: All four Asp251 substitutions had no effect on DEAF1 transcriptional repression activity of the DEAF1 promoter, however, p.Asp251Asn displayed increased repression ($p < 0.05$). Compared to WT-DEAF1, p.Asp251Asn ($p < 0.001$), p.Asp251Ala ($p < 0.05$), and p.Asp251Gln ($p < 0.001$) substitutions significantly impaired DEAF1 transcriptional activation of the Eif4g3 promoter. Both p.Asp251Ala and p.Asp251Gln impaired DNA binding to all three DEAF1 ligands. In contrast, p.Asp251Glu and p.Asp251Glu mutations exhibited varying affinities for all three ligands. DEAF1 p.Asp251Glu bound to both 6 and 9 bp CG-spacings similarly to wildtype

(WT), and p.Asp251Glu showed preferential binding to 9 bp and, to a lesser extent, 11 bp CG-spacings.

Conclusion: These findings indicate that Asp251 of the KDWK binding motif facilitates binding to flexibly spaced DNA ligands, and has provided insights regarding the effects of Asp251 variants on DEAF1 transcriptional function at 2 different promoters. Variants at Asp251 might result in differential transcriptional regulation of specific genes in comparison to WT-DEAF1.

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Abstract 1702

Structure-function characterization of KAP1/TRIM28 requirements for HIV-1 transcription and latency reactivation

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One of the main roadblocks of HIV-1 eradication is the persistence of a latent reservoir despite treatment of infected individuals with highly suppressive anti-retroviral therapy. As such, defining the molecular mechanisms governing cycles of latency establishment, maintenance, and reactivation will illuminate novel targets for alternative eradication strategies. Previous studies in the lab have proposed the host factor KAP1/TRIM28 activates latent HIV-1 when CD4+ T cells are exposed to stimulation (McNamara et al., Mol Cell 2016 and Morton et al., Cell Rep 2019); however, the mechanisms remain poorly understood. Given this gap in knowledge, the main goal of my project is to define KAP1 domains and functions required for latent HIV-1 reactivation. While addressing this goal, two studies reported that KAP1 is a repressor of HIV-1 (Maura et al., PNAS 2019 and Ma et al., eLife 2019). To settle on this controversy, we devised multiple genetic assays in various cell models of latency to further interrogate whether KAP1 is an activator or repressor. Collective evidence suggest KAP1 is indeed a transcriptional activator, and not a repressor, consistent with prior studies from our lab (Randolph et al., Frontiers 2022; Randolph and D'Orso, in preparation). Having validated KAP1 activator function, we are currently working on defining protein interfaces and specific functions required for HIV-1 transcription stimulation. We will discuss data concerning the importance of KAP1 multi-domain architecture and RING E3 ubiquitin ligase. Overall, this project will contribute to our understanding of mechanisms regulating HIV-1 proviral latency and reactivation.

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ASBMB

Abstract 1711

Intersection of one-carbon metabolism and mitochondrial genome maintenance

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Mitochondria contain their own DNA (mtDNA), which encodes 13 subunits necessary for oxidative phosphorylation. The nuclear genome encodes all other mitochondrial proteins. Nearly 60 nuclear-encoded proteins are associated with mtDNA in nucleoid complexes, but the role of nucleoid proteins in regulating the mitochondrial genome is unclear. Interestingly, a group of proteins involved in one-carbon and redox metabolism, including methylenetetrahydrofolate dehydrogenase 1 like (MTHFD1L) and serine hydroxymethyltransferase 2 (SHMT2), have been assigned as nucleoid proteins. MTHFD1L creates formate for transport to the cytoplasm, where it is used for purine synthesis. SHMT2 catalyzes the cleavage of serine to glycine, synthesizing 5,10-methenyltetrahydrofolate, an important coenzyme in the synthesis of amino acids and nucleotides. We hypothesize that nucleoid proteins MTHFD1L and SHMT2 may relay nutrient status signals to control mtDNA maintenance and expression. MTHFD1L and SHMT2 were overexpressed in HeLa cells. DNA, RNA, and protein were isolated 24 hours post-transfection. Western blot analysis confirmed overexpression of MTHFD1L and SHMT2. Quantitative PCR was used to analyze mtDNA content and transcript levels to ascertain the effect of MTHFD1L and SHMT2 overexpression on mtDNA replication and transcription. No significant change in mtDNA content was observed. MTHFD1L overexpression increased mitochondrial transcription, while SHMT2 overexpression had little effect. MTHFD1L and SHMT2 were knocked down in HeLa cells. RNA was isolated at 24 hours post-knockdown. Quantitative PCR was used to analyze transcript levels to ascertain the effect on mtDNA transcription. MTHFD1L and SHMT2 knockdown decreased mitochondrial transcription at 24 hours, although a loss of cell viability was also observed. We conclude that SHMT2 levels do not significantly impact mtDNA transcription. MTHFD1L levels impact mtDNA transcription. Ongoing efforts are focused on understanding the molecular mechanisms underlying transcriptional changes in response to changes in MTHFD1L levels. It is possible that driving the expression of MTHFD1L leads to an increase in purine levels required for RNA synthesis.

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Abstract 1723

Cell Based Studies on the Effect of POLRMT and MRPL12 Post-Translational Modifications on Mitochondrial Protein Synthesis

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Mitochondria play an essential cellular role in the production of adenosine triphosphate (ATP) through the process of oxidative phosphorylation. To synthesize ATP, mitochondria rely on their own genome that encodes 13 protein subunits of the electron transport chain. The regulation of mitochondrial transcription is not well understood. We hypothesize that post-translational modifications (PTMs) of two proteins involved in mitochondrial transcription may alter their function and serve as a way to regulate gene expression in response to the nutrient status of the cell. Using mass spectrometry PTMs, including lysine acetylation and serine/threonine phosphorylation, were identified on the mitochondrial RNA polymerase (POLRMT) and ribosomal protein L12 (MRPL12), a protein that binds and stabilizes POLRMT. Site-directed mutagenesis was performed to create MRPL12 and POLRMT mutants mimicking these PTMs (acetylation mimic (lysine to glutamine) and phosphorylation mimic (threonine to glutamate). POLRMT and MRPL12 mimics were overexpressed in HeLa cells. DNA, RNA, and protein were isolated 24 hours after transfection to determine mtDNA content, transcript level, and protein abundance, respectively. The transcript levels varied upon overexpression of POLRMT PTM mimics, while mtDNA content was unchanged except for POLRMT K402Q and T993E. Western blot analysis showed POLRMT was successfully overexpressed. CYTB protein levels slightly decreased in HeLa cells transfected with POLRMT mimics. A slight decreasing trend in transcript levels of mitochondrial-encoded genes was observed upon overexpression of MRPL12 PTM. To determine if PTMs of POLRMT and MRPL12 vary in response to the nutrient status of the cell, we induced the short-term loss of available glucose coupled with the addition of pyruvate and glutamine, growth conditions which have been previously shown to increase oxidative metabolism. DNA and RNA were isolated at multiple time points to analyze mtDNA content and transcript level, respectively. Transcript and mtDNA levels trend higher with increasing nutrient deprivation time.

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Abstract 1751

How Promoter Sequence in rrnBP1 and λPR Promoters Affect Transcription Start Site Selection and Other Initiation Kinetics

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The goal of this research was to determine how differences in the discriminator regions of different rRNA promoters affect transcription start site (TSS) selection as well as overall transcription kinetics. The promoter region of DNA is the sequence that allows RNA polymerase to bind and form an open complex (OC), and contains several regions. From furthest upstream of the TSS to closest, these different elements are the UP element, the −35 region, the −10 region, and finally the discriminator. The promoter also includes a spacer region between the −10 and −35 elements which sometimes contains an extended −10 element. These regions are recognized by the RNAP to open a single-stranded bubble around the TSS, called an open complex. The rrnBP1 promoter is a well studied rRNA promoter. The spacer of this promoter is unusually short at just 15 bp, while the discriminator is unusually long at 8 bp. In contrast, the λPR promoter is much more stable and has a consensus spacer length of 16 bp and a discriminator of 6 bp. The unique sequence of rrnBP1 likely contributes to the instability in the rrnBP1 OC, as the length of the discriminator may cause prescrunching of the DNA in the OC and higher instability before transcription begins. λPR contains a 6 bp discriminator that is believed to be the ideal length for accommodation in the OC. Recently, we studied initial transcription from various promoter models, termed R(R), L (L), and L(R). L(L) and R(R) are both synonymous with λPR and rrnBP1 promoters, respectively, although all constructs contain the initially transcribed region (ITR) of λPR. L(R) is a hybrid of the two promoters and has the entire sequence of λPR but the 8 bp rrnBP1 discriminator sequence. Primer extension, wherein the length of a nascent RNA strand is determined by PAGE, performed on the different primers reveals that R(R) has a relatively constant start site at the expected position of +9 (i.e. 9 positions after the end of the − 10 sequence). However, L(R) frequently starts at +6 in addition to the expected +9. This was determined by running various samples of L(R) and R(R) through an acrylamide gel to separate the different DNA strand lengths, with longer strands having an earlier start site. Length of the strands were controlled by altering the sequence so that the first C was at +17, and cytidine triphosphate (CTP) was withheld so that only the strand could only be transcribed up to +16. Experiments performed with L(R) and R(R) found that replacement of the rrnBP1 discriminator with the λPR sequence to make L(R) had a large impact on the TSS selection in the L(R) promoter compared with R(R). Thus, upstream components of the promoter, such as the spacer, are key components to TSS selection with a longer discriminator. This is especially true in the case of a normally unfavorable 8 bp promoter found in rrnBP1, but seems to be accommodated for by the shorter spacer. Further research is being conducted to determine what parts of the spacer region of promoters affect TSS selection and OC stability. We have designed three new promoter sequences that contain components of the rrnBP1 spacer region (either extended −10, the region between the extended −10 and the −35, or both). These sequences and their results will yield further information about how the sequence of promoters affect their TSS selection and OC stability.

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Abstract 1781

VEZF1 is an insulator binding protein that promotes cardiac mesoderm differentiation by activating WNT signaling

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Epigenetic regulation in conjunction with signaling pathways govern cellular differentiation and mammalian development. Vascular endothelial zinc finger 1 (VEZF1) is a transcription factor that is expressed predominantly in the mesoderm of a developing mouse embryo. The mesoderm generates progenitor cells that form the cardiovascular system (heart and blood vessels) post-gastrulation. Previous studies have established the role of Vezf1 as an insulator binding protein, with a potential to regulate chromatin conformation genome-wide. Based on its expression profile, we hypothesized that Vezf1 has a critical role in development of the cardiovascular system. Using murine embryonic stem cells (mESCs), we sought to investigate the early role(s) of VEZF1 in cardiomyocyte differentiation. We show that genetic ablation or conditional knockdown of Vezf1 in mESCs impairs cardiomyocyte differentiation, as determined by the absence of contractile activity in these mutants. The absence of cardiomyocytes from Vezf1 mutant cell lines was confirmed using gene expression analysis and immunofluorescence. We observed a strong reduction of mesodermal cells in the Vezf1 mutants despite small molecule stimulation of WNT signaling, suggesting a potential role of VEZF1 in regulating the WNT signaling pathway. Indeed, our gene expression analysis and ChIP-qPCR indicate that VEZF1 associates with the promoters of WNT signaling genes to activate their expression. Mechanistically, we identified that in absence of VEZF1, the insulator binding protein, CTCF, binds opportunistically to repress the expression of WNT signaling genes. These studies point towards a model where VEZF1 regulates the formation of cardiac progenitor cells by activating the WNT signaling pathway. Our observations suggest a central role of VEZF1 in determining the fate of mesodermal cells into cardiovascular progenitors, the absence of which impairs cardiomyocyte differentiation. This study is the first to delineate the functional role of VEZF1 in the early development of cardiomyocytes and may form the foundation for future cardiac regenerative medicine.

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Abstract 1792

Recruitment of Transcriptional Regulators by RNA polymerase II Promotes Phase Separation during Eukaryotic Transcription

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The recent discovery of cellular liquid-liquid phase separation emerges as a new membraneless organelle functional in many biological processes. Various condensates can coexist in the same biological pathway without apparent interference with each other. One critical question yet to be fully addressed is how different condensates prevent phase fusion while the same biomolecule enters, exits, and migrates to different functional condensates. Here, we investigate the intrinsically disordered region of RNA polymerase II and identify what biophysical forces lead to the phase transition. The reversible phosphorylation of the CTD by enzymatic activities of kinases/phosphatases dissolve/reform condensates due to the negative-charge repulsion, where the location or the identity of the negative charge groups have little impact. While the phosphorylation of RNA polymerase II allows its ejection from the transcriptional initiating condensates, its interaction with proteins specifically recruited to phosphoryl-CTD forms a new kind of condensate correlating with productive transcription. Intriguingly, the condensates formed at different stages of transcription vary in their physicochemical properties, allowing them to remain separated without fusion even when located right next to each other. The different governing biophysical interactions can be converted with enzymes modifying the non-covalent interactions. In cells, Pol II molecules with different charge states are recruited to various biomolecular condensates. Our results support a model that transcription regulators and factors are sorted automatically in different transcription condensation states in cells based on the governing biophysical forces and their interaction with the core proteins of the condensates.

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Abstract 1802

Testing Sequence Dependence of Rate Constants of Transcription Initiation

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RNA polymerase (RNAP) is the central enzyme responsible for the process of transcription by locating promoters in a genome, opening nearby DNA to form an open complex (OC), and initiating the transcription of nascent RNA. Using rapid quench flow (RQF) experiments, we can determine the kinetic rate constants for the incorporation of nucleotides by *E. coli* RNAP and, by altering the DNA template sequence and/or solution conditions, determine the factors that affect the rate of transcription, especially in the first 10–12 nucleotide incorporations after which promotor escape occurs. Previously, we have used this technique to measure initial transcription from the λPR promotor. From these data we hypothesize, based on variation in rate constants, that promotor escape occurs in three distinct steps. However, recent data showing that positions incorporating GTP tend to have a higher rate constant than those incorporating UTP have suggested that the variation between steps could be due to the sequence being transcribed, rather than breakage of promotor contacts. To ascertain the sequence dependency of initial transcription, we are measuring transcription from the previously used DNA template with GTP and UTP incorporation sites swapped. If the rate constants in the mutated template are similar to those in the original, despite the changes in encoded NTPs, then the data will support the hypothesis that the process of transcription is sequence dependent. However, positions that now incorporate GTP having higher rate constants than those that now incorporate UTP would suggest the variation is largely due to sequence. In these transcription experiments pre-formed OC is mixed with initiation solution (which includes NTPs) and allowed to react for an amount of time (0.1–120 seconds) before being quenched. RNA in the timepoints are then separated via PAGE, which allows us to observe the different band lengths at single-nucleotide resolution and quantify the RNA produced per open complex over time. These RNA species are then quantified and fit with a kinetic mechanism to generate rate constants. The data from our initial experiments show that with a low concentration of GTP transcription from the mutated template behaves more similarly to the original sequence with a low concentration of UTP. This suggests that the sequence plays a factor in the rate of transcription, but rate constants for the mutated template cannot be determined without measuring transcription in the presence of low UTP concentration. Once determined, the rate constants will make it clear if steps of initial transcription are more controlled by the properties of the promotor and open complex or by the sequence of the ITR.

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Abstract 1803

MEIS2 regulates BACE1 expression in Alzheimer's disease models via transcriptional activation

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Background: As the major pathological hallmark of AD, amyloid plaque is caused by the imbalance between amyloidogenic and nonamyloidogenic pathway of amyloid precursor protein (APP). BACE1 (beta-site amyloid precursor protein cleaving enzyme 1) cleavage of APP is the rate-limiting step for Aβ production and plaque formation in AD. Down-regulate the expression or activity of BACE1 could effectively reduce Aβ concentrations in human individuals and AD animal models. Despite previous researches have revealed extensive achievements about BACE1 regulation in AD, the exact biological machinery about transcriptional regulation has still to be discovered.

Methods: We examined the MEIS2 expression of cerebrospinal fluid (CSF) and serum samples in different stages of AD by ELISA. Using the APP/PS1 mice overexpressed or knocked-down of MEIS2 by AAV stereotactic microinjected, we observed the cognitive function affected by MEIS2 via Morris water maze (MWM) test and the novel object recognition (NOR) tasks. For *in vitro* studies, we used Aβ1–42 co-cultured and APPswe-transfected to establish AD cell models and utilized molecular biological approaches such as western-blot, RT-qPCR and ELISA to study gene expression. The chromatin immunoprecipitation (ChIP) and luciferase reporter gene assay were used to investigate the mechanisms of transcription regulation.

Results: In this study, we found that MEIS2 was notably elevated in mild cognitive impairment (MCI) stage and dementia of the Alzheimer type (DAT) stage with CSF and serum samples. Interestingly, CSF MEIS2 level in MCI-stage was higher than that in DAT-stage (953.3 ± 64.45 pg/mL at MCI-stage and 816.9 ± 59.55 pg/mL at DAT-stage). The MWM and NOR tests showed that down-regulation of MEIS2 could improve learning and memory retention of AD model. MEIS2 knockdown also decreased the number of amyloid plaques and the levels of Aβ1–40, Aβ1–42 and sAPPβ in AD mice. *In vitro*, Aβ1–42 and sAPPβ levels increased significantly after infected with AAVMEIS2 in the culture medium of mouse primary neurons. The HT22 and N2A cell lines were used to further confirm the results above. In contrast, down-regulation of MEIS2 markedly decreased the levels of products of APP amyloidogenic cleavage pathway. Moreover, MEIS2 was related with the expression BACE1 in the hippocampus and cortex tissues at different ages in APP/PS1 mice and age-matched controls. The simple linear regression showed a positive correlation between the protein levels of MEIS2 and BACE1. Similar results were observed in two model cells of AD. According to ChIP and luciferase reporter gene assay, the

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MEIS2 binding-site was been identified at −1410 bp to −1395 bp to TSS region of Bace1 gene promoter.

Conclusions: Together, our findings suggested that MEIS2 bind to the Bace1 promoter to regulate BACE1 expression by transcription pathway. Despite Bace1 gene regulatory network has complex mechanisms, a profound study of transcription pathways provides a new idea for revealing the pathological mechanism of AD and a way to improve AD patients' cognition in early stage.

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Abstract 1808

A Budding Yeast Model to Study Oncohistones Defines Roles for H3K36 Acetylation that May be Linked to R-loop Resolution

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In eukaryotes, DNA is wrapped around histones to form highly organized chromatin. Local packaging of the DNA by histones contributes significantly to regulation of gene expression. Although histones are essential proteins, somatic missense mutations in histone genes have been linked to cancer and these specific variants are referred to as "oncohistones." However, how single amino acid changes alter histone function to drive cancer phenotypes is not known. Histone proteins are extensively post-translationally modified so these oncohistone changes could impact the landscape of post-translational modifications. Thus, a single amino acid change in a histone protein can disrupt PTM patterns. To explore oncohistone mutations and potential alterations in PTM patterns, our lab has employed a budding yeast model system which has only two copies of histone H3 whereas humans have 15 copies of the histone H3 gene. The budding yeast system allows us to create cells that express the oncohistone protein as the sole form of histone H3. Previous research done by our group has focused on the chondroblastoma-linked H3K36M mutation and revealed that a change to H3K36, such as H3K36R and H3K36M, confers sensitivity to formamide. Formamide alters RNA metabolism and leads to the formation of R-loops, a three-stranded nucleic acid structure composed of both DNA and RNA. Although R-loops play a role in gene regulation, accumulation of these structures can pose a potential threat to genome integrity. Cells that express either H3K36M and H3K36R as the sole form of histone H3 show growth defects on formamide; however, deletion of SET2, which encodes the histone methyltransferase that specifically targets H3K36, does not confer slow growth on media containing formamide. This result suggests that loss of H3K36 methylation does not underlie the formamide growth phenotype. Thus, we hypothesize that perhaps the loss of acetylation at H3K36 may cause formamide-sensitive growth. To test this hypothesis, we deleted the GCN5 gene, which encodes the Histone Acetyl Transferase (HAT) that acetylates a number of lysines on histone H3 tails. Our results demonstrate that loss of GCN5 confers sensitivity to formamide. Consistent with our hypothesis, a GCN5ΔSET2Δ double mutant exhibits the same growth phenotype on formamide as cells lacking GCN5. We speculate that together with other H3 lysine acetylation, H3K36ac may coordinate with other PTMs such as phosphorylation of H3S10 (H3S10ph) to recruit factors that facilitate R-loop resolution. Our current model is that H3K36ac may be downstream of H3S10ph, a signal indicating R-loop formation, facilitating recruitment of homologous recombination factors to resolve these R-loops. Overall, this study can

provide insight into the crosstalk between histone PTMs and resolution of R-loops to prevent genome instability, a hallmark of cancer. Through these studies, we can explore the underlying mechanisms by which H3K36M drives oncogenesis and potentially provide insight into therapeutic targets for H3K36M-linked chondroblastoma.

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Abstract 1912

Retinoid x Receptor Alpha DNA Binding for Mitochondrial Transcription

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Mitochondria are important organelles in most eukaryotic cells due to their essential role in metabolism. Within the matrix of the organelle, they also have their own genome (mtDNA) which encodes many of the protein subunits for complexes within the oxidative phosphorylation (OXPHOS) pathway. The remaining OXPHOS protein complex subunits are encoded in the nuclear genome, translated in the cytosol and imported into mitochondria. Transcriptional coordination between these two genomes to produce functional OXPHOS complexes is critical to maintaining cellular homeostasis. This process likely requires regulatory proteins/transcription factors that determine the expression of these genes. Retinoid X receptor alpha (RXRα) is a protein that has many functions in human cells and is a ligand activated nuclear receptor. It can also translocate to the mitochondria where its purpose is unknown. The goal of this project is to determine the role of RxRα as a mitochondrial transcription factor. We have expressed and purified the RXRα protein from bacteria for use in an *in vitro* mitochondrial transcription assay. Using an electrophoretic mobility shift assay (EMSA) we have also shown the purified protein binds to DNA with canonical binding sequences (DR1 repeats) and spacing as well as non-canonical spacing and have concluded RXRα binds as a dimer with a single nucleotide space between the binding sites being optimal. We have applied these results to assess changes in mitochondrial transcriptional output based on RXRa binding. Our aim is to investigate RxRa binding the mtDNA using atomic force microscopy (AFM). In doing so we will better understand the mechanisms that are involved in mtDNA transcription. If RxRα is a transcription factor that is another protein that we can regulate to increase or decrease transcription. Being able to increase or decrease transcription in mitochondria is important as mitochondrial dysfunction causes a wide array of diseases and plays a part in aging.

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Abstract 1939

Exploring the Potential Role of Histone H2A Proteins in Bdelloid Rotifer DNA Repair

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Bdelloid rotifers are a class of microinvertebrates that have the capability to repair extensive DNA damage caused by desiccation. However, the mechanism for this type of repair is unknown. Chromatin (DNA and histone proteins) regulates DNA repair in many eukaryotes. Interestingly, the seven histone H2A proteins in the bdelloid *Adineta vaga* have C-terminal tails longer than those found in other animals, which could implicate these proteins have a role in DNA repair. Our project's objective is to determine which of the histone H2A genes in *A. vaga* may be involved in DNA repair. Firstly, we used 3′Random Amplification of cDNA Ends (3′RACE) to amplify, clone, and sequence the 3′UTRs of the seven H2A genes. In eukaryotes, H2A genes that have polyadenylated mRNA are associated with cellular functions that can include DNA repair. Conversely, the mRNAs of core histone H2A genes are not polyadenylated and instead possess a histone downstream element (HDE) and stem-loop in the 3′UTR. Thus, polyadenylated H2A genes that we found using 3′RACE were the strongest candidates for genes involved in DNA repair. Secondly, by inactivating the H2Abd3 gene using CRISPR genome editing we further investigated the potential role it plays in DNA repair. We designed and amplified a single guide RNA (sgRNA) to direct Cas9 to the target sequence within the gene. We also designed a single-stranded deoxyoligonucleotide (ssODN) template to introduce mutations via homology directed repair following Cas9 cleavage. Using an *in vitro* Cas9 cleavage assay, we verified that the sgRNA/Cas9 complex cut the H2Abd3 target site. We carried out CRISPR by introducing the sgRNA/Cas9 complexes and the ssODN template to *A. vaga* embryos via electroporation. Then, PCR screening and deep amplicon sequencing for the desired mutation in CRISPR-treated rotifers was done to determine if CRISPR was successful. We will conduct desiccation recovery assays on mutants to determine if H2Abd3 influences DNA repair. This will help us understand this extraordinary DNA repair system in these unusual animals and broaden our knowledge beyond a select number of model organisms.

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Abstract 1949

SPDEF modulates transcriptional output to regulate multiple pathways: Immune and Inflammatory pathways as potential hubs targeted by SPDEF in RCC 7/T cells, a line of Prostate cancer cells of African American origin

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Sweaty Koul, Hari Koul

Metastatic Castration Resistant Prostate cancer (mCRPC) results in over 30000 deaths annually in the USA. There are no curative therapies for patients suffering from mCRPC. Moreover, African American men are twice as likely to die from Prostate cancer (PCa) as compared to Caucasian American men, however, the underlying mechanisms for such disparity are not fully understood. SAM pointed domain-containing Ets transcription factor (SPDEF) is a DNA-binding transcription factor known to play roles in prostate gland by targeting sets of genes via binding to 5′-GGAT-3′ DNA sequences. We have previously identified that SPDEF plays an anti-metastatic role in cell-based as well as mouse models. We observed that in RCC7/T cells, a prostate cancer cell line of the African American Origin, SPDEF expression is lost and that there was a significant decrease in cell migration and invasion, plus regulation of genes associated with metastasis such as EMT markers following stable expression of SPDEF. In an attempt to clarify the underlying mechanisms, we opted to analyze the downstream changes in SPDEF-expressing cells at the transcriptional level via RNA-seq. To achieve this, we purified total RNA samples in triplicate from both SPDEF- and Vector control-RCC7/T cells after confirming the overexpression by immunoblotting. The RNA quality control, cDNA library preparation, and sequencing was performed at Louisiana Cancer Research Center (LCRC) using in-house NGS device. The data were received as fold-changes, and p value of less than 0.05 was considered significant. In order to have an insightful interpretation of this complex omics data and to find the significantly altered molecules and pathways changed by SPDEF overexpression, we used the QIAGEN Ingenuity Pathway Analysis (IPA) web-based software. Our data revealed increases in multiple pathways including hypercytokinemia/hyperchemokinemia (21 molecules, 25% match), interferon signaling (13 molecules, 35% match), and pathogen recognition receptor in infectious disease pathogenicity (17 molecules, 15% match). The IPA analysis of disease- and functional-related changes suggests significant upregulation of genes associated with inflammatory responses, hematological system development of function, immune cell signaling and trafficking, and cellular movement. Our network prediction analysis also highlights the central role of the inflammatory cytokine IL-6 and inhibitor of NFKB (NFKBIA). Interestingly, when overlaid with the publicly available prostate signaling pathway, our data showed a unidirectional increase in NFKBIA and HSP90 which control

the cell survival and AR signaling, respectively. Collectively, these data suggest immune and inflammatory pathways as potential hubs modulated by SPDEF in prostate cancer with special emphasis in African American Men. Further studies are under progress to functionally test these pathways and their correlation with anti-metastatic role of SPDEF in PCa.

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Abstract 2027

The regulatory binding dynamics of N-acetylglucosamine Repressor, NagC in *Vibrio cholerae*

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Swagata Ghosh

N-acetylglucosamine (GlcNAc) present at the infection sites of the pathogen acts as a multifaceted signaling molecule apart from being a nutrient source affecting the survival and virulence in both prokaryotic and eukaryotic microbes. In our earlier study, in an enteric bacteria *Vibrio cholerae*, we have shown that global repressor NagC acts as both positive and negative regulators of gene expression involved in various cellular processes in addition to GlcNAc catabolic gene expression. NagC was hypothesized to manifest a novel mode of gene regulation by coordinating with the redox state of the cell through its interaction with GMD (GDP mannose 4, 6-dehydratase) that binds to NADP/NADPH. Further, it becomes intriguing to study the molecular details and regulatory dynamics involved in the NagC controlled gene expression globally. In the current study, we have developed ChTAP protocol by creating a strain engineered for NAGC expressing TAP (Tandem Affinity Purification) tagged fusion protein (NagC-6XHis-3XFLAG). We have performed genome wide ChTAP on CHIP and time lapse microscopy studies to determine global *in-vivo* binding patterns of NagC in presence of both glucose and GlcNAc. Interestingly the results revealed that in contrast to previous studies, NagC constitutively associates with nucleoid under both glucose vs GlcNAc exposed conditions which could be an added advantage for the organism to utilize carbon sources simultaneously and to avoid wastage transcription during On-Off switch. For future studies intended to unveil the 'active vs idle' binding status of transcription factor (TF) NagC, we are adapting 'competition ChIP' by creating strain expressing two copies of TF; one copy with TAP tagged NagC (6XHis-3XFLAG) expressing under native promoter and other with GFP tagged NagC (NagC-GFP) expressing under arabinose inducible promoter. These studies can provide new insights into understanding of the fundamental logic of gene regulatory mechanisms in prokaryotes. This in turn will open up avenues for the development of therapeutic reagents for combating bacterial infections through prevention of a successful colonization by modulating the functioning of global TFs.

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Abstract 2076

Development of a Model System to Evaluate the Effects of CTCF on Global Transcriptional Patterns during Cell Differentiation

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Chromatin architecture has been shown to be important for the establishment and maintenance of transcriptional patterning during cell differentiation and development. One of the major players in establishing higher-order chromatin structure is CCCTC-binding factor (CTCF), a ubiquitously expressed and highly conserved gene found extensively across eukaryotic organisms. This gene has specifically been noted for its intrinsic roles in genomic imprinting, topological domain organization, and DNA loop structuring. When mutated, repressed, or knocked out, CTCF is embryonic lethal and thus, it has been classically challenging to study the role of this critical epigenetic regulator in transcriptional programming. Work presented here describes the establishment of an inducible CTCF knockdown model in both HeLa and human mesenchymal stem cells (hMSCs) using the recently established CRISPRi/a system. Treatment of genetically-modified clonal cell lines demonstrates robust GFP and RFP reporter gene expression after 48 hours. Results indicate stable integration and robust expression of the CRISPRi/a system within these newly established lines. Ongoing experiments continue to evaluate the efficacy of this knockdown model on the ability of HeLa cells to respond to environmental stress and the capacity of hMSCs to differentiate into chondrocytes or adipocytes in the absence of CTCF.

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Abstract 2082

Genetic knockout of two putative copper sensing transcription factors in *Pseudogymnoascus destructans*

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The infectious disease known as White-nose syndrome (WNS) is caused by the filamentous fungus *Psedogymnoascus destructans* (Pd) and has caused the rapid collapse of many cave-dwelling North American bat populations since it was first detected in 2006. As keystone species, bats play vital roles in our natural ecosystems and assist in agricultural productivity. Currently, there are no approved treatments for WNS, and it is unclear how Pd adapts and thrives on its' bat host. Transcriptional profiling at active fungal infection sites suggests that there is a battle over trace metal ions at the host-pathogen interface. There is a general lack of understanding of how copper and other trace metal ions participates in *Pseudogymnoascus destructans* host colonization and how they contribute to WNS disease. The purpose of our work is to understand the basic cell biology of *P. destructans* towards extremes in copper bioavailability. We observe that Pd can survive over a wide range of extremes in Cu-bioavailability ranging from <1 μM to >1 mM in synthetic media. We have identified two putative copper-sensing transcription factors (hypothetical genes VC83_01625 and VC83_00821) which contain a classical N-terminal copper binding-fist domain. We hypothesize that these transcription factors regulate genes associated with cellular Cu homeostasis and may be required for pathogen survival and invasion of its bat host. This poster will present our recent efforts to characterize the Pd Cu-stress response and the development of Pd genetic knockouts of the two putative copper-sensing transcription factors employing *Agrobacterium tumefaciens*-mediated transformation.

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Abstract 2122

Developing an Angelman syndrome model in *Drosophila melanogaster* using tissue-specific CRISPR to knockout Dube3a

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Angelman syndrome (AS) is a neurological disorder occurring in ~1/15000 births and is characterized by cognitive disability, ataxia, seizures, speech impairment, and a happy disposition with sporadic bursts of laughter. AS is caused by a loss-of-function or deletion mutations in the maternal allele of UBE3A, a gene that encodes an E3 ubiquitin ligase. Drosophila UBE3A, or Dube3a, is 77% identical to the human protein, making *Drosophila melanogaster* a powerful genetic model for AS studies. Although a mutation model was previously developed, Wu et al 2008, the two alleles from this original study, specifically Dube3a15b, have genetically drifted and are no longer homozygous viable. In addition, AS is caused by a complete loss of UBE3A function in neurons, but the Dube3a15b allele may still make some product hindering the potency of AS specific experimentation. Our goal was to use new tools available in Drosophila to produce an improved AS model via a complete excision of the Dube3a gene using a tissue-specific CRISPR-Cas9 system. Moreover, using this system, we will make Dube3a loss of function excisions in somatic tissues (neurons and glial cells) to observe which tissue-specific deletions lead to ataxia, a key phenotype of AS. We have not yet achieved a successful Dube3a knockout in the germline, but we continue to perform the crossing scheme to capture the moment when the Cas9-gRNA system successfully creates Dube3a deletions in fly gametes. We have observed significantly slower climbing ability of flies with a ubiquitous deletion (actin-Cas9) and a glial cell (repo-Cas9) specific deletion of Dube3a compared to control flies. We are still collecting data for climbing rates of flies with neuronal-specific deletions (elav-Cas9).

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Abstract 2126

Epstein-Barr Virus Reactivation Mediated *in vitro* by Short-Chain Fatty Acids

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Kelly Gorres

Epstein-Barr Virus (EBV) causes infectious mononucleosis and was one of the first human viruses linked to a variety of cancers. EBV lives in two lifestyles, a latent and a lytic life cycle. Expression of the viral immediate-early gene BZLF1 triggers the latent/lytic switch in the virus. Targeting this switch is important for preventing spread by keeping the virus from reactivating. Sodium butyrate (NaB) activates the expression of BZLF1 gene and serves as an HDAC inhibitor in the cells. We have observed that short-chain fatty acids, propionate and valerate, also induce BZLF1, but not as potently. However, branched medium-chain fatty acids such as valproate and valpromide inhibit BZLF1 expression in Burkitt lymphoma cells. A source of naturally occurring short chain-fatty acids can be found in the metabolic pathways of amino acids. To determine the effects of other short chain-fatty acids, leucine metabolite 4-methyl-2-oxovalerate was tested for effect on EBV-positive Burkitt Lymphoma cells. Using RT-qPCR, the effects of these molecules on BZLF1 expression were measured both accompanied by NaB and without. The administration of 4-methyl-2-oxovalerate had no observed activation of the viral lytic cycle. When introduced with NaB, this metabolite showed a slight increase in activation of BZLF1 compared to NaB alone. Investigations of the effects of other short-chain fatty acid metabolites on EBV reactivation and histone acetylation are compared. Discovering regulators of EBV will aim to understand the natural viral life cycle and to promote the development of clinical solutions for EBV related diseases.

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Abstract 2129

Approaches to Increased Spatiotemporal Resolution of Chromatin Immunoprecipitation Techniques through the Employment of Nonconical Amino Acid Histones

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Our lab utilizes an *in vivo* crosslinking technique to examine histone-protein interactions in the living nucleus of yeast. Most recently, we mapped the contacts through which the RSC remodeler ATPase motor subunit, Sth1, interfaces with nucleosomes, and detailed the binding regulation via histone acetylation and SUMOylation. Our approach provided temporospatial details from the view of the histone protein encoded with the crosslinking probe (p-benzoylphenylalanine, pBPA), however, while we can provide a clear map of Sth1 binding to the nucleosome we cannot assess details of where along the chromatin fiber this binding is occurring. One important aspect of our research is that we do not remove genomic copies of endogenous histone genes and crosslinking histones compete for nucleosomal occupancy. Because of this, it is difficult to evaluate where along the chromosomal fiber the crosslinked remodeler is acting. To address this issue, we are utilizing chromatin immunoprecipitation (ChIP) and developing a double crosslinking and double immunoprecipitation technique. Prey proteins of interest are expressed from a genetic background producing Myc-tagged variants (Sth1-myc), while the crosslinking bait histones are HA-tagged. Following histone-Sth1 crosslink capture, standard ChIP protocols are used. An initial HA-precipitation of histone associated DNA fragments allows for purification of the crosslinking histone and isolation of DNA in these fragments. A secondary IP from the Myc-tag on the Sth1 clarifies the original pool to identify the bridging interaction. We have successfully managed to perform the double IP and generate a pool of DNA that is associated with Sth1-histone protein-protein interactions. These were verified by following fractions of protein via western blotting. We are currently identifying appropriate qPCR targets to assess the efficacy of our double-ChIP protocol. Understanding the global incorporation of the pBPA-histones alone would be of great importance to this work. Additionally, this approach could be a powerful tool to assess nucleosome occupancy of proteins that interact at the nucleosome more transiently and are difficult to pull down in normal coprecipitation reactions.

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Abstract 2175

Identification of YAP1-specific distal enhancers controlling ARPC5 expression in melanoma

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The actin nucleating complex Arp2/3 (actin-related protein 2 and 3 complex) is essential for cellular physiology, and is involved in cell migration, cell morphology, and DNA repair. However, dysregulation of Arp2/3 is involved in pathological disorders and is linked to cancer progression and metastasis. Melanoma, the most severe of the skin cancers, has a high mortality rate due to metastasis. Metastatic melanoma is characterized by cytoskeleton regulation changes and increased invasive potential. We have previously discovered that the transcriptional co-activator YAP1 (Yes-associated protein 1) promotes cell invasiveness and metastasis in cancers by driving the expression of ARPC5, a component of the ARP2/3 complex. YAP1 inhibition caused immediate downregulation of ARPC5, evidence that ARPC5 is a direct YAP1 downstream target. YAP1 lacks DNA binding domains and exerts transcriptional control through other transcription factors, thereby complicating the discovery of genes directly regulated by YAP1. To better understand how YAP1 regulates ARPC5, we utilized in silico analysis of SK-MEL-5 HiC data mapping potential enhancer elements distal to the ARPC5 gene promoter. We identified 51 potential enhancers and reduced this number to ten through phylogenetic footprinting using multiple species sequence alignments. To confirm in-silico findings, we utilized Cleavage Under Targets & Release Using Nuclease (CUT&RUN) assays. Data from Next Generation Sequencing (NGS) of CUT&RUN extracted DNA was trimmed, aligned, and YAP1-specific peaks mapped against the ten enhancer sequences, reducing this number to three. Motif analysis of those three potential enhancer sequences identified several candidate transcription factor recognition motifs, revealing several possible YAP1-specific binding partners. This analysis of potential ARPC5 regulatory enhancers will reveal genetic elements that regulate the important cytoskeletal regulatory complex (ARP2/3) and influence invasive potential in melanoma, as well as uncover YAP1-specific transcriptional binding complexes involved in ARPC5 expression. Furthermore, our method outlined here has the potential to allow simplified mapping of distal enhancer sequences back to the individual genes they control.

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Abstract 2259

The 3′ Region of the ZPA Regulatory Sequence (ZRS) is Necessary for Activity and Contains a Pivotal E-Box Binding Site

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Charmaine Pira, Kerby Oberg

Sonic Hedgehog (Shh) directs anterior-posterior limb development and is secreted from the zone of polarizing activity (ZPA). The ZPA regulatory sequence (ZRS) is a highly conserved, limb-specific enhancer that regulates Shh. ZRS microdeletion prevents Shh expression, while single nucleotide variations (SNVs) within the ZRS cause ectopic Shh expression and polydactyly. This study's objective is to elucidate the ZRS mechanism of action by characterizing the ZRS regulatory subdomains and identify keying transcription factor binding sites. Hoxd13, Hand2, and Twist1 are transcription factors (TFs) that regulate Shh in the limb and the ZRS contains putative binding sites for each: two Hoxd13 sites and three E-boxes, sites bound by basic helix-loop-helix (bHLH) TFs like Hand2 and Twist1. Further, reports indicate these factors act through the ZRS: Hand2 purportedly binds the ZRS through a central E-box, Hand2 and Twist1 regulate Shh as homo- or heterodimers, and both Hand2 and Hoxd13 activate the ZRS *in vitro*. We hypothesized that the central ZRS region and E-box are required for activity and that other E-boxes and Hoxd13 sites localize ZRS activity to the distal posterior limb mesoderm. To determine ZRS subdomain function, we generated three ZRS fragments (5′, central, and 3′) by progressive digestion and made paired fragment constructs to test subdomain interplay. Additionally, we altered five putative binding sites, alone or in concert, using site-directed mutagenesis. All ZRS constructs were cloned into a GFP reporter. We electroporated plasmids into the presumptive limbs of Hamburger-Hamilton stage 14 chicken embryos, harvested 48hrs post-transfection, and imaged via fluorescence microscopy. We found the 3′ fragment is necessary for ZRS activity, while the 5′ and central fragments have no activity alone or when paired. In combination with the 3′ fragment, the 5′ fragment increases 3′ fragment activity, while the central fragment appears to dampen 3′ fragment activity. We also found that simultaneous mutation of all five binding sites abrogates ZRS activity. We then discovered reinstating each of the Hoxd13 sites can restore minimal, focal activity. Restoring the 5′ and central E-boxes had little effect, but the most 3′ E-box is sufficient for robust activity even in the absence of the other binding sites. This was surprising as the central E-box was reported as the likely Hand2 binding site. Our data suggest the ZRS 3′ region is necessary for activity and contains the 3′ E-box that Hand2 likely uses to regulate Shh. We also found the Hoxd13 binding sites may influence localized activity within the ZPA. This study demonstrates that ZRS is composed of different regulatory modules and identifies key binding sites. Future steps will clarify which TFs act through the key sites and the role of bHLH dimerization in ZRS function.

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ASBMB

Abstract 2262

Deletion of Potential Chromosome Segregation Gene in *Streptomyces coelicolor*

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Streptomyces coelicolor is a gram-positive bacterium located in the soil, commonly used to study antibiotic production and as a model for multicellular prokaryotic development. *S. coelicolor* has a complex life cycle, forming filaments with multi-genomic compartments leading to single linear chromosomes within mature sporular compartments. In this bacterium, chromosomes segregate through the ParABS system. ParA and ParB proteins organize the subcellular space to play a role in chromosome segregation. While ParA and ParB play a major role in chromosome segregation, mutations of each gene only contributed to small phenotypic growth changes. Using transposon mutagenesis, a novel mutant was previously identified in our lab with a chromosome segregation defect using propidium iodide staining with fluorescence microscopy. The mutated gene resulted in 22% anucleated spores compared to the less than 1% anucleated spores found in wild type. The gene was found to have a transmembrane spanning region, so it may participate in chromosome segregation by tethering the DNA to the membrane. Orthologues of this novel gene were found to be highly conserved in *Mycobacterium tuberculosis* and *S. venezulae*. Extending this study into another species, propidium iodide staining with fluorescence microscopy was used on an *S. venezuelae* single deletion strain for the novel gene ortholog and a double deletion mutant of the novel gene ortholog and adjacent gene positioned in a potential operon. Preliminary results showed the *S. venezuelae* single mutant and the wild type had 0.78% anucleated spores, whereas the double mutant had 1.18% of anucleated spores. Both mutants displayed shorter sporulated chains in comparison to wild-type *S. venezuelae*. The subtle chromosome segregation phenotype with a noticeable difference in the lengths of sporular chains in *S. venezuelae* may indicate a species difference pertaining to this gene's role. Currently, the novel gene is in the process of being deleted in *S. coelicolor* by using REDIRECT. The deletion mutant will be compared to the wild-type and transposon insertion strains. This study will allow us to determine if the identified gene is the cause of the *S. coelicolor* chromosome defect or whether the strain should undergo whole genome sequencing to identify the gene of interest. In addition, the deletion of the novel gene will lead to further understanding of the chromosome segregation process in filamentous bacteria.

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Abstract 2266

Aromatic Residues Dictate the Transcriptional Repressor and Single-Stranded DNA Binding Activities of Purine-Rich Element Binding Protein B

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Robert Kelm, Jr.

Purine-rich element binding protein B (Purβ) is a single-stranded DNA (ssDNA) and RNA binding protein that functions as a transcriptional repressor of genes encoding certain muscle-restricted contractile proteins in the setting of cellular stress or tissue injury. A prior report from our laboratory implicated specific basic amino acid residues in the physical and functional interaction of Purβ with the smooth muscle-alpha actin gene (*Acta2*) promoter. Independent structural analysis of the related protein Purα uncovered a role for several aromatic residues in the binding of Purα to ssDNA. We examined the functional importance of a comparable set of hydrophobic residues that are positionally conserved in the repeat I (Y59), II (F155), and III (F256) domains of murine Purβ. Site-directed Y/F to alanine substitutions were engineered and the resultant Purβ point mutants were tested in various biochemical and cell-based assays. None of the mutations affected the cellular expression, structural stability, or dimerization capacity of Purβ. However, the Y59A and F155A mutants demonstrated weaker *Acta2* repressor activity in transfected fibroblasts and reduced binding affinity for the purine-rich strand of an *Acta2* cis-regulatory element *in vitro*. Mutation of Y59 and F155 also altered the multisite specificity of Purβ for ssDNA and diminished the interaction of Purβ with Y-box binding protein 1, a co-repressor of *Acta2*. Collectively, these findings suggest that some of the same aromatic residues, which govern the specific and high affinity binding of Purβ to ssDNA, also mediate certain heterotypic protein interactions underlying the *Acta2* repressor function of Purβ.

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Abstract 2273

Sex and genotype affect mouse hippocampal gene expression in response to mild blast-induced traumatic brain injury

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Blast-induced traumatic brain injury (bTBI) has been identified as an increasingly prevalent cause of morbidity and mortality in both military and civilian populations over the past few decades. In military training and combat zones, bTBIs are most often mild and may result from exposure to blast waves from a variety of sources. Long-term neurodegenerative effects including cognitive impairments can develop without effective diagnosis and treatment. Additional attention is needed to better understand how genetic predispositions may affect response to bTBI at the molecular level by influencing susceptibility to damage vs. resilience and repair. We used a well-established blast tube system mimicking pressure waves experienced during a field explosive detonation. Whole transcriptome sequencing (RNA-Seq) was performed with eight genetically distinct mouse strains to assess the effects of sex and genotype on sub-acute gene regulation at 30 days post-injury following a single mild/moderate (180 kPa) blast exposure. We evaluated changes in gene ontology categories for cellular components, molecular functions, and biological processes and conducted pathway analysis to determine significantly affected canonical biochemical pathways and compared these results between sexes and across strains. We identified pathways where differential expression across strains overlapped between sexes (i.e., CREB signaling, G-protein coupled receptor signaling) as well as pathways that were more significantly dysregulated in one sex (i.e., S100 family signaling in females). We also found that certain strains tended to have more variability in differentially expressed genes between sexes. Additionally, several strains showed overlapping GO enrichment profiles related to mitochondrial genome maintenance and processes related to cellular metabolism. This study represents a multi-level examination of how certain genotypes may influence response to bTBI and provides a foundation for the identification of therapeutic targets that could be modulated to improve the health of Veterans and others with histories of blast exposures.

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Abstract 2308

Microscopy-based detection of DNA methylation at a specific genomic locus: Epigenetic Visualization Assay with automated image acquisition and analysis

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Detecting DNA methylation at a specific genomic locus in individual cells has been challenging. Recently, a microscopy-based Epigenetic Visualization Assay (EVA) was developed to detect DNA methylation at a specific genomic locus by using fluorophore-labeled DNA oligonucleotides and anti-5-methylcytosine (5mC) antibody. EVA is based on an in situ proximity reaction that generates fluorescent signals proportional to DNA methylation levels at a genomic locus of interest. In EVA, fixed cells on a glass coverslip are heated to denature DNA, and gene-specific oligonucleotides with a tether sequence hybridize to the target genomic locus. The 5′-phosphorylated oligonucleotide containing a red fluorescent dye binds to the tether sequence, thereby visualizing the position of the target locus in a nucleus. The oligonucleotide containing a green fluorescent dye binds to part of the red fluorescent oligonucleotide, resulting in two-color fluorescence at the locus. When DNA is methylated at the target sequence, anti-5mC antibody associated with alkaline phosphatase (AP) is recruited to the site. AP dephosphorylates the red fluorescent oligonucleotide, protecting it from subsequent lambda exonuclease digestion. When DNA is not methylated at the locus, the 5′-phosphorylated red fluorescent oligonucleotide is partially digested by subsequent lambda exonuclease treatment, removing green signals but retaining red signals. Nuclei are stained with NucBlue (Hoechst 33342). The ratio of green to red fluorescence signal intensity is calculated as a measurement of DNA methylation levels at the target locus. EVA allows us to quantitate DNA methylation levels at a specific genomic locus for individual alleles within each nucleus. However, acquiring fluorescent images manually with a 100x oil-immersion objective is time-consuming. Here, we improved EVA by using automated microscopy and image analysis. Automated fluorescence microscope with a 20x or 40x air objective captured images efficiently at the resolution sufficient for foci detection. Automated image analysis software identified NucBlue-positive areas as nuclei and red-positive areas within nuclei as the target genomic loci and measured green and red signal intensities for each red focus. We calculated the ratio of green to red signal intensity as DNA methylation levels at the EGR1 locus in the SK-MEL-2 human melanoma cell line. Automated image acquisition and analysis minimized hands-on time and significantly shortened time required for EVA. The improved EVA is suitable for quantitating allele-specific DNA methylation levels at a specific genomic locus especially when the number of cells is limited.

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Abstract 2313

Na+/H+ exchanger 5 (NHE5) protein is a potential target for therapeutic interventions in altered Male Reproductive Physiology

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Joe Powers, Paul James

Na+/H+ exchanger 5 (NHE5) is a protein that regulates intracellular pH. It is one of the six NHE proteins, also intracellular pH regulators, found in mammalian testis and sperm. An increase in sperm intracellular pH facilitated by NHE proteins is an event critical for fertilization to occur. However, the specific role NHE5 plays in this process, and reproduction remains unclear. This study aims to understand the distinct role of NHE5 in reproduction, testis, and sperm physiology using the Mus musculus male mice model. Protocol involving animal use was reviewed and approved by the IACUC. We targeted the disruption of NHE5 protein expression in the testis of male mice using CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) Cas9 gene editing technology, thus, generating a gene knockout (KO) mice model. Polymerase Chain Reaction (PCR) analyses on KO mice testis tissue samples (sample size, n = 3) revealed a deletion in portions of gene and messenger RNA (mRNA) sequences compared to control mice. Interestingly, the sequence deletions observed did not alter the reproductive capability of KO mice compared with control mice. Further studies using immunoblotting, cell culture, and spermatogenic techniques will investigate changes in NHE5 protein expression and function and how these changes impact the morphology/physiology of KO mice testis and sperm cells. Although our KO mice models remain fertile in our study, additional studies will provide more robust insights into NHE5 role in male reproductive physiology and allow us to draw a solid conclusion. Increasing efforts to study testis/sperm proteins associated with reproduction will improve our understanding of male reproductive physiology and enable the discovery of novel, safer therapeutic targets for treating male reproductive physiology deficiencies.

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Abstract 2338

A trans-HAT mechanism via HDAC3 acetylation allows GCN5 to regulate p300 sites

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While crosstalk between modifications of different histone lysine residues has been extensively studied, the impact of crosstalk between different histone-modifying enzymes, particularly HATs and HDACs, on the ability of specific HATs to regulate histone modifications remains poorly understood. Here, we report a trans-HAT mechanism that allows GCN5 to regulate p300-specific lysine sites by acetylation and inactivation of HDAC3. We show that GCN5 physically associates with HDAC3 and acetylates HDAC3 at K44 and K49 in the N-terminus of HDAC3, a region important for activation of HDAC3 by inositol tetraphosphate (IP4) and the deacetylase interaction domain (DAD) of nuclear receptor corepressors. Biochemical and functional studies show that HDAC3 acetylation by GCN5 prevents HDAC3 activation by preventing IP4-dependent interaction between acetyl-HDAC3 and DAD. The K44K49/QQ mutation recapitulates the effect of GCN5. We also show that HDAC3 can deacetylate not only histones but also p300, thereby inactivating p300. Although H3K27 is a p300-specific site, by inactivating HDAC3, GCN5 acquires the ability to regulate p300-dependent H3K27 acetylation by permitting and enhancing p300-mediated acetylation, representing a double-negative mechanism. *In vivo* analysis of ChIP-Seq and RNA-Seq results provided further evidence that GCN5 has the ability to control p300 sites (H3K27/K18) at genes colocalized with HDAC3 but not at genes bound by GCN5 alone. We propose that this trans-HAT mechanism is important for GCN5 to regulate transcription by controlling both canonical and non-canonical GCN5 sites.

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Abstract 2354

RNA Polymerase II transcription attenuation at multiple genes in yeast depends on the mRNA 3′-end processing factor Hrp1 and its RNA recognition motif

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Premature termination of transcription (i.e. attenuation) is an ancient and widespread form of gene regulation, spanning all three domains of life and viruses. The significance of premature termination is evidenced by attenuation defects linked to cancer, viral infection, developmental abnormalities, and neurodegeneration. Attenuation of eukaryotic RNA Polymerase II (Pol II) transcription is more prevalent than once thought, but its mechanism of action is poorly understood at most gene targets. Pol II attenuation is best studied in the yeast model eukaryote *Saccharomyces cerevisiae*, where it was originally discovered to occur via the Nrd1-Nab3-Sen1 non-coding RNA termination pathway. More recently our lab characterized a "hybrid" attenuation pathway involving Sen1 along with the Hrp1 cleavage factor from the mRNA 3′-end processing complex. How widespread is Hrp1 activity in Pol II attenuation, and how does Hrp1 function at different regulatory targets? In this study, we used a genetic selection to characterize the recognition elements of several Hrp1-dependent attenuators (MNR2, RAD3, and SNG1). Attenuator readthrough mutants clustered in conserved regions of the mRNA 5′-UTR, including putative Hrp1 consensus binding sites. We used site-directed mutagenesis to alter the Hrp1 RNA-recognition motif (RRM) at a highly conserved amino acid F162, comparing F162A, F162H, and F162W substitutions. The hrp1 RRM mutants in single copy exhibited slow-growing or lethal growth phenotypes, and in a heterozygous context we observed variable dominant negative attenuator readthrough defects. Our ongoing experiments are testing more direct interactions of the Hrp1 RRM with various attenuator RNAs using a yeast three-hybrid system, dissecting the sequence-dependency of attenuator recognition. Overall, these results expand the "hybrid" Pol II attenuation pathway, confirming it is Hrp1-dependent and likely involves direct Hrp1 recognition of attenuator elements near the 5′-end of mRNA targets.

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ASBMB

Abstract 2372

A Novel Role of mRNA Decapping Enzymes in Repression of Pervasive Transcription in Yeast

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Pervasive and stochastic transcription has been well documented in many eukaryotic genomes. In yeast, around 85% of the whole genome is transcribed. It remains an open question about whether pervasive transcription is just biological noise or has some distinct functional roles. How pervasive transcription is controlled also remains largely elusive. Decapping enzymes are known to play a critical role in controlling coding transcript stability via mRNA decay. However, the general regulatory mechanism remains unclear. In the budding yeast *Saccharomyces cerevisiae*, the Dcp2 protein is one of the primary components of decapping enzyme complex regulated by decapping activators, including the Dhh1 and the Pat1. The XRN1 gene encodes the 5′-3′ exonuclease family that interacts directly with the decapping complex to dominate subsequent mRNA degradation. In this study, we profiled the transcription initiation landscape using nAnT-iCAGE for yeast strains with knockout of these decapping enzymes and XRN1 (dcp2Δ, dhh1Δ, pat1Δ and xrn1Δ). We found that deletion of decapping genes lead to significant increase of transcription start sites (TSS), especially in dcp2Δ, which generates ∼40% more TSS clusters (TCs) compared to its wild type. Most of these mutant-specific TCs are found in non-canonical promoter regions, such as protein-coding regions and intergenic regions, suggesting a novel role of Dcp2 in repressing pervasive transcription initiation in yeast. We further found that the repressive role of Dcp2 might be mediated by preferential decapping of transcripts initiated from non-canonical promoter regions as well as repressing transcription initiation from these regions. This study uncovers a novel regulatory role of Dcp2 and contributes to a better understanding of repressive mechanisms of pervasive transcription initiation.

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Abstract 2391

Determining the Effects of Poly-A Tracts and Monovalent Cations on Nucleosome Unwrapping Equilibrium

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Dan Grilley

Nucleosomes, composed of 147 base pairs of DNA wrapped around an octamer of histone proteins, play a role in gene regulation and cellular function by controlling access to the genetic information stored within DNA. The placement and movement of nucleosomes along strands of DNA is determined, in part, by the presence of homopolymeric stretches of deoxyadenosine nucleotides on one strand of double stranded DNA, commonly referred to as poly-A tracts, which are overabundant in eukaryotic genomes. The poly-A tracts exclude nucleosomes in a manner that depends upon the purity and length of the A-tract. We have previously shown that A-tracts adopt unique structures that are preferentially stabilized by specific monovalent cations. The exclusion of nucleosomes by these long A-tracts, greater than 15 base pairs, impacts the accessibility of nearby DNA. The equilibrium accessibility of DNA within the nucleosomes, determined by unwrapping and rewrapping rates, is also an important factor in regulating DNA dependent processes. The impact of short A-tracts, 6–8 base pairs, on nucleosome dynamics is poorly understood. Using competitive reconstitution and equilibrium FRET measurements in the presence of different monovalent cations, we have investigated how the length and placement of short A-tracts within the nucleosome affects the formation and equilibrium accessibility of the nucleosomes. We demonstrate that the same cations that stabilize the unique A-tract structure exacerbate the effects of A-tracts on nucleosome stability and equilibrium accessibility.

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Abstract 2474

Tagging and Expression of *Candida albicans*

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Allison Swain

Candida albicans live as harmless commensals in different part of human body. The overgrowth of these organisms will lead to disease. In addition, a main goal of *C. albicans* research is to investigate response of multiple cells to the environment in the human host, in particular to enable colonization and infection. *C. albicans* is distantly related to *Saccharomyces cerevisiae* and they share conserved genes at the mating type locus (MTL). However, there are distinctions between the mating cycle of these two species. The *C. albicans* mating cycle involves concerted chromosome loss instead of meiosis, and they must undergo a phenotypic switch from to white- to opaque in order to mate. In *C. albicans*, IME4 regulates white-opaque switching, however where the IME4 mutant has more stable opaque cells than wild type (WT). The IME4 has been tagged with mNeon green. A Generated IME4-mNeon green expression reveled protein expression are specific to MTLa and MTLalpha cells whereas as MTLa/alpha cells protein expression not detected. The generated IME4-mNeon expression will be used to localize the key m6A sites and functional outcomes involved in regulating white-opaque switching and *C. albicans* using flow cytometry and immuno preceptation. Also, IME4-mNeon green will be used be used to analyze how IME4 and m6A responds various environmental signals to mediate changes in metabolism using switching assay protocol.

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Abstract 2475

How Do the MyoD Binding Regions Upstream and Downstream of the Acta1 Gene Influence Transcription of a-actin mRNA?

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Gigi Beas, Gilberto DeSalvo, Sandra Sharp

The transcription factor MyoD contributes to myogenesis (muscle development) by binding to conserved regulatory sequences typically found upstream of target genes. Interestingly, binding sites for MyoD have been found downstream as well as upstream of Acta1, one of MyoD's target genes. The role of the downstream binding regions is unknown. I hypothesize that MyoD binding both upstream and downstream of Acta1 is necessary for developmentally regulated, muscle-specific Acta1 transcription. Testing this hypothesis will increase our knowledge of longer-distanced, downstream transcriptional regulation, and will provide regulatory mutations to test for in ACTA1-related myopathies that have not been ascribed to a coding-region mutation. I am performing transient plasmid transfections with normal and mutated Acta1 regulatory regions inserted upstream and downstream of a firefly luciferase reporter gene. The constructs are transfected into mouse pre-muscle and fibroblast cells, and a dual luciferase assay is performed at both myoblast (undifferentiated) and myocyte (differentiated) stages. I have determined the quantity of normalizer NanoLuc plasmid to use; have found that apparent NanoLuc activity is lower in growth versus differentiation conditions; and have observed an apparent effect of the specific plasmids present in the transfection mixture on NanoLuc activity. I address how these findings will inform our data analyses. I have performed transient transfections and luciferase assays on constructs containing the 5′, 5′ and various 3′, truncated 5′, mutated 5′, mutated 5′ and various 3′, truncated 5′ and various 3′ binding sites, and empty constructs. My results showed that normalized Acta1 promoter-driven firefly luciferase expression is higher in the presence of the upstream MyoD binding region than in its absence in myocytes, but not in myoblasts, consistent with previous reports. Additionally, constructs containing both the 5′ and 3′ binding sites, showed higher transcriptional activation when compared to constructs containing just the 5′ binding site. Constructs with mutated or truncated 5′ but intact 3′ binding sites also showed higher transcriptional activation when compared to constructs containing just the mutated or truncated 5′ binding sites in myocytes only. So far, these results show that the 3′ binding site may play a role in increasing expression of Acta1 mRNA. Experiments using the empty constructs also showed that there is no cryptic promoter affecting the amount of Acta1 transcription in either myoblasts or myocytes. More trials with these constructs, as well as experiments using a non-muscle fibroblast cell line to show cell specificity are underway.

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ASBMB

Abstract 2477

Exploring the interaction of DNA damage and transcription in the initiation of adaptive mutagenesis in *Saccharomyces cerevisiae*

Tina Saxowsky, *Pacific Lutheran University*

Mechanisms by which non-dividing cells can acquire adaptive mutations in responses to stress or selective pressure is an active field of study. Multiple mechanisms related to DNA damage and genome maintenance have been shown to contribute in various systems, but the role of DNA base damage, and specifically its influence on transcription in this process, has not been explored fully. Many types of base damage can be bypassed by RNA polymerase in mutagenic fashion, resulting in a pool of mutant mRNAs and potentially a population of mutant proteins that could alter the phenotype of the cell. We hypothesize this set of steps could be an important first step in adaptive mutagenesis, as the mutant protein generated could allow cells to resume DNA replication and convert this damage to a permanent and heritable mutation. Using a forward mutation assay for canavanine resistance in *Saccharomyces cerevisiae*, we can assess both replicational mutation rates in dividing cells and adaptive mutation frequencies in growth-arrested populations using a variety of knockout strains. Not surprisingly, compromising the cell's ability to repair the prevalent oxidative lesion 8-oxoguanine (*ogg1Δ*) increases both replicational mutation rates (7-fold) and adaptive mutation frequencies (3-fold) over WT indicating that persistent DNA damage affects both processes. To further implicate transcription, we knocked out the gene for Transcription Factor IIS (TFIIS), which facilitates the ability of backtracked RNA Polymerase II to cleave the nascent transcript, realign the RNA end in the active site, and resume transcription. *In vitro*, including this protein in transcription assays reduces transcriptional mutagenesis past 8-oxoguanine. We removed this gene both alone (*dst1Δ*) and in combination with *ogg1* (*dst1Δogg1Δ*). While removing this protein had no appreciable effect on replicational mutagenesis in either context, and *dst1Δ* by itself did not increase adaptive mutation frequencies, the double knockout strain displayed a further increase in adaptive mutagenesis (nearly 2-fold) over just the *ogg1Δ* strain, indicating that an increase in steady state DNA damage in combination with a decreased ability of transcriptional proofreading combined to increase adaptive mutation frequency. We also explored the strand bias of the DNA damage leading to mutation, as our hypothesis predicts that adaptive mutations arise because of DNA damage in the template strand relative to transcription. We find this to be true, with ~80% of adaptive mutations following this trend. Unexpectedly, the replicational mutants sequenced as a control display ta similar strand bias for the transcriptional template strand, although the mutation sites are largely nonoverlapping, and the sequence context around the lesion appears to differ between replicative and adaptive mutants. Current efforts focus on exploring these differing sequence contexts to better understand the different mechanisms at work in replicational and adaptive mutagenesis.

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Abstract 2596

A pair of RNA binding proteins inhibit expression of an ion transporter to maintain lifespan in *C. elegans*

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Olivia Marx, Adam Norris

Little is known about how post-transcriptional mechanisms regulate the aging process. Previous studies have established that the RNA binding double mutant exc-7; mbl-1 has a strong lifespan defect. Our goal is to investigate the underlying mechanisms by which exc-7 and mbl-1 RNA binding proteins control lifespan and healthspan. Here we show that the nervous system is the primary tissue in which exc-7 and mbl-1 expression is required for normal lifespan. RNA-seq analysis identifies that there are many unique gene dysregulation and splicing event changes in the exc-7; mbl-1 double mutants and that a number of the genes identified through RNA-seq analysis have effects on lifespan. We systematically characterize these genes, and find one gene whose upregulation is important for the exc-7; mbl-1 lifespan phenotype: nhx-6, a potassium:proton and sodium:proton antiporter. nhx-6 is highly upregulated in the exc-7; mbl-1 double mutant, and mutation of nhx-6 partially rescues the lifespan and some healthspan defects in the exc-7; mbl-1 double mutant. Thus, we have identified a pair of RBPs which inhibit the expression of nhx-6, an ion transporter, to maintain lifespan in *C. elegans*. nhx-6 expression increases with age and decreases with dietary restriction. Together these results indicate that not only does nhx-6 partially rescue the lifespan and a number of healthspan phenotypes seen in the exc-7; mbl-1 double mutant but that nhx-6 expression is also associated with aging processes in wild-type worms.

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Abstract 2644

Mediator kinase disruption links altered myometrial stem cell enhancer dynamics with uterine fibroid formation

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Claire Sun, Lindsey Barron, Robert Schenken, Nicholas Stansbury, Shin-Fu Chen, Kuang-Lei Tsai, Ron Firestein, Thomas Boyer

Recurrent somatic mutations in the RNA polymerase II transcriptional Mediator subunit MED12 are dominant drivers of uterine fibroids (UFs), accounting for 70% of these clinically significant lesions. Within the multiprotein Mediator, MED12, along with MED13, CycC, and CDK8 (or its paralog CDK19) comprise a 4- subunit kinase module. Previously, we and others have shown that MED12 is an obligate activator of CycC- CDK8/19 and that UF driver mutations in MED12 alter CDK8 T-loop stabilization and impair Mediator kinase activity. These studies aim to identity Mediator kinase substrates and their potential pathogenic contribution to cellular transformation and UF development. Using a SILAC-based quantitative phosphoproteomics workflow coupled with a highly specific chemical inhibitor of CDK8/19 (CCT251545), we identified Mediator kinase substrates in myometrial stem cells (MM SCs) derived from consented UF patients' tissue using protocols approved by institutional review board. We obtained 166 proteins with significantly decreased phosphorylation upon CDK8/19 inhibition, including 71 nuclear proteins representing high-confidence Mediator kinase substrates. Orthogonal validation confirmed a selected subset of these proteins, including the myogenic SC transcription factor FOXK1, to be direct targets of MED12-dependent CDK8 phosphorylation in a manner abrogated by the most common UF driver mutation in MED12 (G44D), implicating these substrates in disease pathogenesis. Complementary transcriptome-wide profiling of Mediator kinase-inhibited MM SCs revealed alterations in cell cycle and myogenic gene expression programs to which Mediator kinase substrates, including FOXK1, could be linked. Accordingly, we hypothesize that MED12 mutation-induced Mediator kinase disruption drives MM SC transformation through perturbation of the core transcriptional circuitry, leading to aberrant SCs with altered myogenic and enhanced tumorigenic potential. To investigate the mechanistic basis for SC reprogramming as a function of Mediator kinase activity, we employed global and targeted CUT&RUN sequencing to investigate epigenomic and cistromic changes in Mediator kinase inhibited MM SCs. Preliminary analysis reveals dynamic changes in the super enhancer landscape upon Mediator kinase inhibition. The results of ongoing integrated analysis of epigenomic and RNA expression profiling will be presented in an effort to establish a comprehensive model for MM SC transformation through super enhancer reprogramming and perturbation of the core transcriptional circuitry. Overall, these

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studies identify a new catalog of patho/biologically relevant Mediator kinase substrates implicated in the pathogenesis of MED12-mutant UFs and further provide new insight into the early molecular events that drive MM SC transformation and UF formation.

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Abstract 2687

Nup170-mediated subtelomeric gene silencing occurs via regulation of PCNA levels on DNA

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The nuclear pore complex (NPC), in addition to its role in transporting molecules across the nuclear envelope, also contributes to chromatin organization and gene expression regulation. These latter functions are mediated in part by the inner ring nucleoporin Nup170, which has been implicated in the maintenance of subtelomeric gene silencing. Using a combination of protein-protein interaction, genetic interaction, and transcriptomic correlation analyses, we aimed to uncover the mechanisms whereby Nup170 regulates this process. We found that the Ctf18-RFC complex, an alternative proliferating cell nuclear antigen (PCNA) loader, facilitates Nup170's gene regulatory functions. The Ctf18-RFC complex binds to a subset of NPCs in a cell cycle regulated manner. In the absence of Nup170, PCNA levels on DNA decrease, resulting in the loss of silencing of subtelomeric genes and other normally silenced chromatin. This silencing defect can be rescued by increasing PCNA levels on DNA by removing Elg1, a PCNA unloader. Our findings demonstrate that the NPC mediates subtelomeric gene silencing by regulating PCNA levels on DNA.

This work was supported by grants P41 GM109824 and R01 GM112108 from the National Institutes of Health.

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Topic Category Computational Biology

Abstract 1171

Large-scale transcriptomic analysis identifies novel genes essential for biofilm and rugose colony development of *Vibrio vulnificus*

Hojun Lee, *Seoul National University*

Hanhyeok Im, Duhyun Ko, Dayoung Sung, Sang Ho Choi

Vibrio vulnificus, an opportunistic human pathogen, forms biofilms to survive under host immune defenses and environmental stresses. However, conventional differential expression (DE) analysis of the genes in biofilm and planktonic cells under a single condition has limitations to identify the genes essential for biofilm formation. In this study, a machine learning algorithm named independent component analysis (ICA) was adopted to comprehensively identify the biofilm genes of *V. vulnificus*. ICA analyzed the large-scale transcriptome data of *V. vulnificus* cells under various biofilm and planktonic conditions and then identified a total of 72 sets of independently co-regulated genes, iModulons. Among the three iModulons specifically activated in biofilm cells, BrpT-iModulon mainly consisted of known genes of the regulon of BrpT, a transcriptional regulator controlling biofilm formation of *V. vulnificus*. Interestingly, the BrpT-iModulon additionally contained two novel genes, brpX and brpY. brpX and brpY were shared in other Vibrio species and not yet identified by DE analyses. Genetic and biochemical analyses revealed that BrpT positively regulates the expression of brpX and brpY by binding directly and specifically to their upstream regions. The deletion of brpX and brpY impaired the robust biofilm and rugose colony formation. brpX, predicted to encode a protein carrying calcium-binding repeats, was essential for attachment of *V. vulnificus* to the surface. brpY, predicted to encode a protein containing an acyltransferase-3 domain, was crucial for exopolysaccharide production. In conclusion, ICA identified two novel genes, brpX and brpY, which are regulated by BrpT and essential for the development of biofilms and rugose colonies of *V. vulnificus*.

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Abstract 1193

Further understanding of transcriptional regulatory networks of enterohemorrhagic *Escherichia coli* via independent component analysis

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Hojun Lee, Dayoung Sung, Sang Ho Choi

Transcriptional regulatory networks (TRNs) of enterohemorrhagic *Escherichia coli* (EHEC) regulate the expression of the target genes for the pathogens to adapt to various environments. Thus, the understanding of TRNs and their target genes enables the prediction of molecular mechanisms by which EHEC causes disease and survive under host-specific conditions. However, the analyses of current TRNs are still limited to comprehensively understand their target genes generally co-regulated under various conditions regardless of the genetic backgrounds. Here, independent component analysis (ICA), a machine learning-based decomposition algorithm, was performed to decompose the large-scale transcriptome data of EHEC into the modulons, which contain the target genes of several TRNs. The locus of enterocyte effacement (LEE) and the Shiga toxin (Stx) modulons mainly consisted of the Ler regulon and the Stx prophage genes, respectively, confirming that ICA properly grouped the co-regulated genes of EHEC. Further investigation revealed that the LEE modulon contained the Z0395 gene as a novel member of the Ler regulon, and the Stx modulon contained the thi and cus locus genes in addition to the Stx prophage genes. Concurrently, the Stx prophage genes were also regulated by thiamine and copper ions known to control the thi and cus locus genes, respectively. The modulons effectively clustered the genes co-regulated regardless of the growth conditions and the genetic backgrounds of EHEC. The changed activities of the individual modulons successfully explained the differential expressions of the virulence and survival genes during the course of infection in bovine. Altogether, these results suggested that ICA of the large-scale transcriptome data can expand and enhance the current understanding of the TRNs of EHEC.

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Abstract 1195

Mass-action law dynamic theory/algorithm based top-down general bioinformatics

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Two fundamentally different concepts and informatics are discussed for biomedical R&D. The traditional biomedical R&D is an observation-based "Bottom Up" approach, with specific aims, proposal, methods, and feasible conclusions. This is an open approach usually accomplished with multiple experimental evidences and results, using unbiased statistics or other methods to reach a hypothesis, mechanism or conclusions. However, the best-curve-fitting for dose-effect data is frequently empirical and requires many dose-data points. Here, a non-traditional general unified theory-based "Top Down" approach is based on biophysical biochemical fundamental principle of the mass-action law (MAL), which provides features of efficiency, cost-effectiveness, quantitative digital simulation of informatics. This "Top Down" approach used an unprecedented system analysis such as pattern analysis, combinatorial analysis and mathematical induction and deduction, involving the derivation of over 300 reaction-rate equations, and using reverse operation and thinking. This approach led to the discovery of the median-effect equation (MEE) as the unified biodynamics, pharmacodynamics and bioinformatics general principle (BD/PD/BI). The parameter m is dynamic-order signifying shape of dose-effect curve; and Dm, the median-effect dose signifying potency, and the universal reference-point and different dynamic orders common-link. In the MAL-general theory, Dm serves as normalization factor for all doses. The Dm (half affected/half unaffected) is the unified form as Km in enzyme kinetics, Ka in pH-ionization, K in higher-order ligand occupancy, and Kd in receptor binding. MAL principle to multiple entities interactions for dynamics resulted in the general combination index equation (CIE), algorithm, computer simulation which quantitatively determine "Synergism (CI < 1), additive-effect (CI = 1) and antagonism (CI>1) automatically. The MAL-MEE CI theory has been cited over 21 350 times in 1469 scientific journals covering biochemistry, molecular, cell biology and genetics, pharmacology, agricultural, marine, environmental and food sciences. All terms of MEE and CIE are dimensionless relativity ratio thus can be generally applicable in *in vitro*, *in vivo* and in all physical states (in nanoparticles, cells, tissues, organs, animals, humans, diseases, clinical trials, and in environments); in drugs, biologics, radiation, ultraviolet, microwave, thermo-dynamics and photo-dynamics. This MAL integrated unified "Top Down" theory/method is unlikely obtainable from the traditional specific aimed approaches. The theory/algorithm-based "Top-Down" digital approach is opposite and yet complementary alternative to the observation/statistics based "Bottom-Up" traditional approach. Interestingly, the "Top Down" and "Bottom Up" concept, also appear in 4-of-6 Quarks in the model of elementary particles physics, and in Fu-Xi Ba-Gua and Ying-Yang of ancient philosophy that are interacting in pair and complementary to each other. These two opposite approaches share the same ultimate goal of R&D in diversified sciences for unified R&D informatics, in unity, just like two sides of the same coin, or the front and rear views for the same entity. I strongly recommend the MAL-BD/MEE based doctrine of the median (DOM) and the concept of unity of the bio-R&D informatics be utilized for integrated translational medicine and for the digital precision medicine.

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Abstract 1320

Single Molecule Theory of F1-ATPase

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Matthew Anderson, Kaitlin Snodgrass, Nathan Suiter, Sandor Kacso

Steared MD, also known as accelerated MD, explicates the energies required for chemical reactions to occur. Using MD and knowledge of biological processes, one may determine the binding free energy and kinetics of protein-protein interactions (PPI). Proteins traveling with van der Waals forces are considered to travel long ranges in proteins. The potential energies in these proteins exist between bonded and non-bonded orientations. PPIs contain electrostatic and van der Waal potential energies which aid in the forcefield model by adding empirical data to any mathematical function. Steared MD improves sampling by aiding in binding kinetic value discovery, obtaining energy levels, and adjusting the equations for a baseline, ultimately increasing resolution of any calculation. One limitation to MD is that the protonation state or polarizability does not change within a single simulation. Current technologies such as charged-couple device (ccd) cameras are limited in resolution yet prove valuable results for changes in motion. As frames are captured in the motion of a molecule, the ebb and flow of atomic movements proves to be a standard for biophysical research. Using software imaging programs in synchrony with measured imaging and cryo-electron microscopy, mathematical derivations may be made from any atomic movement. With Dr. Volkan and his team paving the way for how physics and biology intersect, their research proves essential tracing molecules in MD. F1 ATPase, an enzyme consisting of 137 743 atoms, contains a rotary motor comprising αβ-dimers which induce torque on its central y-subunit. As this protein structure spins, it fluctuates in trigonometric angles between molecules. Since inorganic phosphates (Pi) are easier to track than the entire protein, it also provides valuable insight on how the motor protein moves molecules and creates catalytic pathways for living organisms. In its potential energy function, Kd represents the force constant of the bond while do represents the individual bond length when the potential energy and all other terms in the function are zero. One example of how a term's energy level is zero is when an atom's harmonic oscillation is at its equilibrium where kinetic energy is at maximum. Juxtapose, its amplitude is where the atom's potential energy is greatest. If another term's additive energy is not zero, its influence on other terms within the function is significant enough to make any datum untrustworthy. We plan to prove the torsional angle of the gamma shaft against the rotors of F1-ATPase. However, the timescales required to simulate these motions can be long in the one to tens of microseconds. We plan to use gaussian accelerated MD to improve sampling of interaction potential energies between communicating proteins, therefore producing the torsional angles of the rotors with respect to the gamma shaft that is currently unknown. An advantage of MD is that it can be used with larger molecules of, for example, half a million atoms. Enzymes of this magnitude contain torsional motion in rotor-like molecules. Volkán Kacsó et al. have assigned me a task in determining the torsional angle of the rotors in F1-ATPase with respect to the central gamma shaft to verify Suiter's results that the angle of rotation is about one hundred degrees. The angle of rotors rotation with respect to rotational velocity has been found to represent a linear function.

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Abstract 1325

Devising Novel Microarray-based Platform for Screening and Sorting Gastruloids

Ian Jan, *University of Washington-Seattle*

Yuli Wang, Adriana Beltrán, Sonja Mihailovic, Samuel Wolff, Jeremy Purvis, Nancy Allbritton

Modeling the critical spatial patterning that occurs during gastrulation *in vitro*, human gastruloids are a powerful tool to study an otherwise inaccessible stage of embryogenesis due to ethical and technical limitations. Specifically, the simplicity and reproducibility of 2D gastruloids enable high-throughput imaging and quantitative analysis of coordinated genetics, cell-cell signaling, and stem cell differentiation. Gastruloids are used to gain insight into the molecular and signaling mechanisms underlying early embryonic development. However, their potential is limited by low-throughput analytical approaches infeasible for large-scale genetic screens utilizing CRISPR-Cas technology. Through a multidisciplinary collaboration spanning from engineering to systems biology, we are integrating innovative technologies into an automated platform that can screen and sort gastruloids in a high-throughput manner. We culture gastruloids on a microraft array, which is a poly (dimethylsiloxane) (PDMS) array of optically clear and releasable polystyrene cell carriers, or "rafts," with embedded superparamagnetic beads for magnetic collection. This device is compatible with reproducible patterning of cells on the raft surfaces and high-quality confocal imaging. Photopatterning Matrigel onto each raft, we geometrically confine H9 human embryonic stem cells and induce differentiation into tissue layers reflecting the germ layers in a developing embryo. The automated raft sorting system images the cell-patterned array, performs sophisticated image processing to detect complex yet subtle phenotypic markers, and subsequently sorts gastruloids for downstream genetic analysis. Currently, we are optimizing both the automated system and the gastruloid culture on the rafts. We plan to screen a library of nearly 300 gene deletions related to the Wnt pathway that regulates gastrulation as a proof-of-concept. Probing the underlying processes of early embryonic development, we can use this platform to better our understanding of pregnancy losses, congenital birth defects, and tissue regeneration.

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Abstract 1363

iCn3D, a Platform to Align and Annotate AlphaFold Structures

Jiyao Wang, *National Library of Medicine*

Thomas Madej, Philippe Youkharibache, Dachuan Zhang, Christopher Lanczycki, Shennan Lu, Gabriele Marchler, Mingzhang Yang, Aron Marchler-Bauer

The vast body of predicted protein 3D structures, computed with AlphaFold and made available to the research community, empowers biologists with the option to visualize aspects of 3D structure for almost every protein of interest. On top of gauging the quality of predictions, researchers will be interested in 3D-structural neighbors, including putative homologs that cannot be detected reliably with sequence-based methods such as BLAST, and site annotations that can now be interpreted in the context of the 3D mapping. The web-based structure viewer iCn3D functions as a platform for comparing (AlphaFold) predicted structures with experimentally determined structures deposited to the Protein Data Bank (PDB), or with other (AlphaFold) predicted structures. A variety of algorithms and methods have been made available, such as VAST (Vector Alignment Search Tool), Foldseek, TM-align, and VAST+. iCn3D also maps annotations onto (AlphaFold) predicted structures, such as conserved domain footprints, functional sites, sites of sequence variation obtained from dbSNP and ClinVar, as well as post-translational modifications. Structure- or sequence-based alignments and annotations are shown via a combination of 3D structure displays, 2D schematic diagrams, and 1D sequence and sequence-tracks displays. Annotations can be exported with Node.js or Python scripts. Users' work can be saved and shared with others via long-lived short links/URLs or iCn3D PNG images, which reproduce the specific custom views and make them available for further interactive analysis. The source code of iCn3D can be found at https://github.com/ncbi/icn3d.

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Abstract 1370

How Selection Bias and Data Interpretation Contribute to Disparities in Health Outcomes and Artificial Intelligence Development

Rosalina Bray, *University of Maryland Eastern Shore*

The use of Artificial intelligence (AI)has been used in clinical prediction and some healthcare related decisions. However, some of the datasets and information used for prediction may be too homogeneous limiting the generalizability of the AI results. Moreover, datasets with limited diversity also contribute to the risk associated with using AI alone in clinical decision-making. The study investigated how selection bias and data interpretation contributes to disparities in health outcomes when using Artificial Intelligence (AI). Clinical and healthcare articles published in PubMED from 2018–2021 on the topic of digital health were reviewed to compare common incidences of bias due to selection. The research determined there are several ways bias attributed to AI can arise. Therefore, when researchers or clinicians are using AI for selection or treatment recommendations, they should consider the inherent bias presented in Artificial Intelligence-based decision support system.

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Abstract 1407

Next generation transcriptomics-based precision oncology

Eytan Ruppin, *Tel-Aviv University*

Precision oncology has made significant advances, mainly by targeting actionable mutations and fusion events involving cancer driver genes. Aiming to expand treatment opportunities, recent studies have begun to explore the utility of tumor transcriptome in guiding patients' treatment. I will describe five new computational approaches that we have developed to this end: First, SELECT and ENLIGHT, that aim to predict patient response from bulk tumor transcriptome and are validated across many different clinical trial datasets. Second, PERCEPTION, which aims to advance precision cancer therapy from single cell tumor transcriptomics. Thirdly, ENLIGHTDeepPT, a precision oncology expression-based approach that starts from tumor histopathological images and successfully predicts response to a broad range of targeted and immune therapies. Fourthly, the development of Liquid-based transcriptomics (LBT) to learn about the tumor immune microenvironment from the blood. Finally, I will discuss the challenges laying ahead.

My research has been funded by the intramural program of the NCI.

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Abstract 1434

Artificial Intelligence in Healthcare: Addressing Disparities, Mitigating Biases & Misinformation

Irene Dankwa-Mullan, *George Washington University; formerly Merative; IBM Watson Health*

Advances in artificial intelligence (AI) and machine learning (ML) technologies hold promise for promoting personalized, patient-centered care and improved health outcomes. The potential of the tools to generate insights from massive amounts of data, in ways that can help inform decisions, interventions, and precision care, is enormous. However, there are growing concerns that use of these technologies may further exacerbate inequities in health, as a result of embedded data and evidence biases. There are also concerns about the ethical implications of these technologies on decision-making and their role in incorporating the critical role of social determinants of health in model development. This presentation will discuss (1) the various sources of bias that gets incorporated into these technologies, (2) how to approach algorithmic or data biases and develop methods to mitigate them, (3) a framework to integrate equity and social justice principles as part of a learning, continually improving health systems, and (4) potential opportunities for research and collaboration to promote equity leveraging AI-ML tools.

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Abstract 1448

A Bioinformatic Investigation of Genetic Insulation Mechanisms in Plants

Asa Laskie, *Fort Lewis College*

Christine Queitsch, Cole Mueth, Josh Cuperus, Kerry Bubb, Sayeh Gorjifard

Climate change threatens food security. Traditional plant breeding relies on parental performance which loses predictive power in rapidly changing environments. Genetic engineering is a promising strategy to generate crops with greater resistance to environmental stresses, improve crop yields, and produce valuable bioproducts made currently with fossil fuels. This strategy is hampered by the lack of diverse, plant derived regulatory elements that drive individual genes in multi-gene cassettes encoding metabolic pathways. The lack of such elements leads to silencing either due to the spread of repressive heterochromatin or the use of the same constitutive viral promoters. Recent research efforts have primarily focused on promoters and enhancers to allow for programmable and tunable expression of transgenes. Here, we focus on insulator sequences, which allow for controlled, often tissue-specific regulation of gene expression and halt the spread of repressive heterochromatin. Unlike in animals, there are few known insulators in plants and their molecular underpinnings are unknown. We use a combination of computational analyses to investigate whether plant genomes contain reoccurring motifs that may function as insulators, as well as where those motifs may exist in the genome. We are also exploring if the insertion of a well-known animal insulator motif and the expression of the protein that it binds will confer insulator activity. Using bioinformatic tools like Find Individual Motif Occurrences (FIMO) and bedtools, we analyzed whether orthologs to CTCF, the principal insulation regulation gene in animals, can be found in the *Arabidopsis thaliana* genome. Our results provide evidence that genetic insulation in plants is not facilitated through CTCF-like orthologs, but perhaps through an entirely different set of insulation genes. To better understand where binding motifs for these insulator proteins might exist in the *A. thaliana* genome, we performed genome-wide analysis of the differences in tissue-specificity (Tau) and expression correlations between adjacent genes in different orientations: head-to-head, tail-to-tail, and head-to-tail. We found that overall tissue specificity was significantly lower in genes in head-to-head and tail-to-tail orientations. We also found that expression correlations between gene pairs in head-to-head and tail-to-tail orientations were significantly lower compared to gene pairs in tandem orientations. Both of these analyses provide us with a hypothesis that insulator motifs are more likely to exist in intergenic regions between genes in head-to-head orientations. Taken together, this research will further our knowledge of insulator function in plants and provide tangible benefits for future engineering of transgenic plants.

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Abstract 1514

The correlation between age, gender, and stage of adenocarcinoma gastric cancer in Brazilian patients

Catarina Rahal, *Tufts University*

Introduction: Gastric cancer is a multifactorial disease that can evolve from both environmental and genetic agents. The purpose of this study was to investigate to what extent age and gender impacted the stage and severity of adenocarcinoma gastric cancer.

Methods: There were 1102 Brazilian patients with adenocarcinoma cancer initially recruited for the study population was narrowed to 490 patients according to treatment susceptibility from 2012 to 2018. Patient data was categorized into five age ranges. Stages of cancer were ranked according to the 7th edition of the TNM gastric cancer staging system. The was analyzed using chi-squared test (significance level of 5%).

Results: The data showed that 39.9% of male Brazilian patients with adenocarcinoma were Stage IV. In female patients, the prevalence was 34.1%. The diagnostic for younger patients (<41.0) in female cases is larger than for the male cases. 10.4% of all female cases are in women who are younger than 41 years old. However, young male patients (<41.0) represent only 3.9% of all male cases. The gender difference was more dramatic in older patients (51years or older), in which male cases seemed to be higher. The p value for gender was 0.348, and the p value for the age group, showing no statistical difference in the stages of the adenocarcinoma gastric cancer in relation to gender or age.

Conclusion: The presence of advanced stages is larger in men than in women. In almost half of the cases of male patients, the cancer had metastasis, was advanced, and survival rate was extremely low. Female patients also had a very low chance of survival. This signifies a small difference of the presence of advanced cases between both genders. Although survival rate seems to be lower in women than men, the number of younger women with adenocarcinoma is larger than in younger men. Given that this Stage IV has a very low survival rate, it can be deduced that men are more likely to die from the disease than women. Adenocarcinoma gastric cancer is more prevelant in older men than women; however, the cancer seems to be more common in young women than in young men. Although there is no statistical difference in the stages of the adenocarcinoma gastric cancer in relation to age or gender, the trends that were established in this investigation should be considered and widely utilized to ensure early prevention, prognosis and intervention of this disease.

It is important to acknowledge the gastric oncology sector from Hospital Santa Casa de Misericordia and Dr. Paulo Kassab, a surgeon from the hospital, who has been my external supervisor and has supported this essay by providing the data to this study.

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Abstract 1605

Differential Pathway Analysis of Early vs. Late Recurrences in ER+ Breast Cancer

Lecheng (Joshua) Lyu, *St. Olaf College*

Robert Clarke, Surojeet Sengupta, Jin Lu

As breast cancer and its recurrence still remain a major global health problem, we sought to identify the common and unique pathways by investigating enriched gene sets in microarray samples that later led to early and late recurrences. We incorporated multiple transcriptomic tumor datasets and statistical tools in our analysis, such as the Gene Set Enrichment Analysis (GSEA). Our results unveil several common and unique pathways as well as genes that may contribute to early (<3 yrs) versus late (>5 yrs) breast cancer recurrence. Our project aims to elucidate patterns and networks among pathways to build predictive models that would open new venues for personalized medicine. In the future, we will investigate additional breast cancer datasets to gain a clearer picture of gene signatures among samples with early and late recurrences. We hope to conduct laboratory experiments to validate the rationale behind the transcriptomics analysis to glean a better understanding of actual cellular networks.

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Abstract 1661

Physics-based screening of AlphaFold and RoseTTAFold predictions on 26 phosphatase proteins

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Taylor Waligora, Jared Ponzetti, Shivam Rama, Suzanne O'Handley

As part of a study on the activity including substrate specificity of enzymes related to extremely drug-resistant human pathogens linked to significant pandemics and epidemics –like *M. tuberculosis* or *S. aureus*, we are interested in computationally determining the structure of a series of 26 Nudix hydrolases or HAD superfamily phosphatases. Although we collaborate to determine these enzyme structures, experiments to determine protein structures are complex and resource intensive. Computational AI-based approaches have recently become a cheap and reliable alternative to experiments. Two of the most reliable and consistent tools available to predict tertiary protein structures are AlphaFold (AF) from Google Alphabet company DeepMind and RoseTTAFold (RF) from Baker's lab. Our goal is to identify between the structures predicted by AI tools for our 26 proteins which one we can trust and which one needs additional work. To this end, we leverage (1) the complementary of the AF and RF approach to protein structure prediction and (2) physics-based molecular dynamics (MD) simulations. First, we predict the structure of all 26 proteins starting from their amino acid sequence using AF and RF. The first screening level is the comparison of the AI prediction for the same protein. Only proteins for which AF and RF agree on the structure move to the next step. The second level of screening tests the physical stability of the predicted structures. The native structure of a protein is its lowest free energy structure, i.e., its most stable structure. To check the strength of predicted structures, we perform long implicit solvent MD simulations, and we monitor how much the proteins tend to change. While this doesn't prove the predicted structure to be the lowest free energy conformation, it weeds out unstable conformations. This double-screening process gives us a reasonable confidence level in the predicted structures' quality. We show the preliminary results of this study. We show that for most proteins, AF and RF predictions agree. We comment on the possible causes of AF and RF prediction mismatches. We finally demonstrate the importance of the physics-screening step. We also comment on potential actions to use physics to recover structure predictions when AF and RF disagree on the protein structure.

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Abstract 1696

Unbreakable Hairpins: Characterizing RNA secondary structures that are persistent after dinucleotide shuffling

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The RNA hairpin, also known as a stem-loop, is a ubiquitous and fundamental feature of RNA secondary structure. A common method of randomizing an RNA sequence is dinucleotide shuffling with the Altschul-Erickson algorithm, which preserves the dinucleotide content of the sequence. This algorithm generates randomized sequences by sampling Eulerian paths through the de Bruijn graph representation of the original sequence. We identified a subset of RNA hairpins in the meta-database bpRNA-1 m that always form hairpins after repeated application of dinucleotide shuffling. We investigated these "unbreakable hairpins" and found several common properties. First, we found that they had on average lower folding energy compared to other hairpins, and frequently contained ultra-stable hairpin loops. Furthermore, we found specific sequence features that restrict the number of distinct Eulerian paths through their de Bruijn graph representation, resulting in a small number of distinguishable dinucleotide-shuffled sequences. Beyond this algorithmic means of identification, these distinct sequences may have biological significance because we found that a significant portion occur in a specific location of 16S ribosomal RNAs, and especially in endospore- forming bacteria. Finally, we present a formula to calculate the number of possible dinucleotide shuffled sequences for an input RNA sequence, which has utility for the general application of the Altschul-Erickson algorithm.

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Abstract 1917

Protein function prediction and classification using deep learning models and knowledge graph mining

Natarajan Kannan, *University of Georgia*

Natarajan Kannan

Proteins perform a diverse array of cellular functions and accurate prediction of their functions is a major challenge in bioinformatics and biotechnology. In this talk, I will present new approaches for protein classification and function prediction using deep learning models, knowledge graph mining and experimental mutational screens. First, I will describe our efforts to build an integrated knowledge graph (KG) conceptualizing information on protein evolutionary relationships, interacting partners, post-translational modifications, pathways, cellular localization and chemical interactions in human and machine-readable format. I will then describe a new scalable graph embedding approach for function prediction using network context in knowledge graphs. I will also describe ongoing projects employing protein language models for sequence classification and function prediction and approaches to augment our predictions with AlphaFold2 models and experimental mutational analysis.

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Abstract 1991

The Low-Cost Marketable Microprocessor Prosthetic Knee by LIMBS International

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David Sandoval, Ethan Ramos

Objective: This work-in-progress result-based paper details a student involved research project in which a fully functional intelligent prosthetic knee with patient adaptation and stumble control will be delivered by 2023. The first phase of the project will detail the design work and iteration done to previous non-optimized iteration of the system. This includes iterations to the frame, hydraulic and electronic systems within the prototype, computer simulated testing and physical electronics testing. The second phase of the project details the manufacturing, assembling and physical testing on amputee patients as well as the market release of the G-Knee 2. A low-cost microprocessor prosthetic knee with a goal weight of 5 pounds, a goal manufacturing cost of $1000, and full stumble recovery and swing control functionality. Methods The materials and methods required for the redesign phase revolve around Lucas Galey's doctoral proposal work. Galey, the executive director of LIMBS had previously delivered a proof of concept prototype for a low-cost MPK (microprocessor prosthetic knee) the G-Knee. The work done this semester is refinement of the previous G-knee system. Software solutions Fusion 360 and Ansys were used to stress test the new hydraulic and frame designs. The electrical system, a ground up design was tested in house with a custom built circuit. Results The current design has a total weight of 3.9 pounds, a cost of $637 and full day of battery power. The team will focus on manufacturing and physical testing on LIMBS amputee patients during the Spring 23′ semester. Finally market research and validation will be conducted before launching the product.

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Abstract 2049

Molecular signatures of post-traumatic stress disorder in war-zone exposed veteran and active duty soldiers

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Post-traumatic stress disorder (PTSD) is a multisystem syndrome. Integration of systems-level multimodal datasets can provide a molecular understanding of PTSD with potential for improved treatment efficacy. Proteomic, metabolomic, and epigenomic assays were conducted on blood samples of two cohorts of well-characterized PTSD cases and healthy/controls: Systems Biology Consortium (340 veterans) and Fort Campbell Cohort (180 active-duty soldiers). All participants had been deployed to Iraq/Afghanistan and exposed to military-service related criterion-A trauma. Molecular signatures were identified from a discovery cohort of 218 veterans (109/109 PTSD +/−). Identified molecular signatures were tested in 82 separate veterans (43/39 PTSD+/−) and in 180 active-duty soldiers (PTSD+/−). Molecular profiles were computationally integrated with upstream regulators (genetic/methylation/microRNAs) and functional units (mRNAs/proteins/metabolites). We identified reproducible molecular features of PTSD including activated inflammation, oxidative stress, metabolic dysregulation, and impaired angiogenesis. These processes may play a role in psychiatric and physical comorbidities, including impaired repair/wound healing mechanisms, cardiovascular, metabolic and psychiatric diseases.

This work was funded through U.S. Army Research Office awards: W911NF-13-1-0376, W911NF-17-2-0086, W911NF-18-2-0056, W911NF-17-1-0069, and from USAMRDC/MOMRP awards: W81XWH-10-1-0021, W81XWH09-2-0044, W81XWH-14-1-0043, W81XWH-10-2-0072 and W81XWH-13-1-0071.

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Abstract 2052

Exploring Experimental PDB Structures and Computed Structure Models from Artificial Intelligence/Machine Learning at RCSB Protein Data Bank (RCSB.org)

Christine Zardecki, *Rutgers University, RCSB PDB*

Paul Craig, Stephen Burley

In addition to >200 000 atomic-level, experimentally-determined, three-dimensional (3D) structures of proteins and nucleic acids archived in the Protein Data Bank (PDB), the RCSB.org research-focused web portal of the RCSB Protein Data Bank (RCSB PDB) provides open access to ~1 million Computed Structure Models (CSMs) generated using AlphaFold2 (from AlphaFold DB) and RoseTTAFold (from Model Archive). Both of these artificial intelligence/machine learning software tools were built on decades of methodological research in de novo protein structure prediction and relied on open access to the immense number of sequences in genomic sequence databases and the experimentally determined structures in the PDB archive. For the avoidance of doubt, experimentally-determined PDB structures and CSMs delivered on by the RCSB PDB are clearly identified as to provenance and reliability. RCSB.org users can query, organize, visualize, analyze, and compare experimental structures and CSMs side-by-side by utilizing powerful tools: - View and Organize

Results: By default, search results are ordered using a query-based relevancy score. Results can be resorted using different criteria (e.g., listing experimental PDB structures first, per-residue confidence score (pLDDT)). - Explore Similar Proteins: "Group" summary pages and search results simplify exploration of PDB structures with similar sequence identity/UniProt ID or were deposited as part of the same study. - Explore Individual Structures: Structure Summary Pages offer details of experimental PDB structures and CSMs. - Assess quality: Analogous to the validation slider for experimental structures, all CSMs report global and local confidence levels as pLDDT scores. - Visualize in 3D: View experimental PDB structures and CSMs in Mol*. Use the standalone Mol* 3D Viewer to upload single or multiple data files, align structures, and run Structure Motif Search. - Download: From Structure Summary Pages, download the ModelCIF data file hosted by the corresponding external archive. RCSB PDB will continue to develop resources to support exploration of experimentally-determined PDB structures alongside CSMs, scaling operations to accommodate growth in the PDB archive and increased availability of predicted structures.

RCSB PDB Core Operations are funded by National Science Foundation (DBI-1832184), US Department of Energy (DE-SC0019749), and National Cancer Institute, National Institute of Allergy and Infectious Diseases, and National Institute of General Medical Sciences of the National Institutes of Health under grant R01GM133198.

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Abstract 2106

Role of 3-O-Sulfation-Induced Rare and Compact Topologies of Heparan Sulfate in Selective Recognition of Proteins

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Umesh Desai, Balaji Nagarajan

Heparan sulfate (HS) is arguably the most diverse linear biopolymer that is known to modulate hundreds of proteins that play key roles in many diseases such as thrombosis, cancer, Alzheimer's and viral infection. We have shown previously that certain sequence-specific 3-O-sulfate groups of heparan sulfate (HS) may induce compact, non-linear, local topologies under dynamical conditions (Comp. Struct. Biotechnol. J. 20 (2022) 3884). We hypothesized such compact, unusual topologies may preferentially recognize HS-binding proteins, which may suggest an evolutionary role of 3-O-sulfation, a modification that remains enigmatic to date. To this end, we screened both linear and compact topologies of HS hexasaccharides against various HS-binding proteins using a novel Semi-Rigid Ensemble Docking (SRED) protocol using GOLD. The SRED protocol, which exhibits superior docking performance in screening vast conformational space of HS oligosaccharides, indicated that some known 3-O-sulfated HS-binding proteins may not only preferentially recognize 3-O-sulfated HS, but also in a noncanonical, compact topology. Overall, this work offers support to the hypothesis that certain 3-O-sulfate motifs in HS are likely to have been evolutionarily selected.

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ASBMB

Abstract 2107

Characterizing influenza virus entry using cryo-electron tomography and convolutional neural networks

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Kangkang Song, James Munro, Celia Schiffer, Mohan Somasundaran

Influenza viruses pose severe threats to global public health. As influenza virions are highly pleomorphic in shape, size, and organization, high resolution structural virology approaches to investigate influenza infection have predominantly been limited to purified viral proteins that do not accurately reflect viral dynamics in situ. In contrast, cryo-electron tomography (cryoET) can depict the 3D organization of pleomorphic influenza and visualize host-virus interactions, but the low signal-to-noise ratio inherent to this technique has limited resolution. Therefore, high resolution investigations are still required to better elucidate crucial stages of the influenza viral life cycle. One such step is influenza entry into the host cell, and the conformational trajectory of the influenza HA glycoprotein during receptor binding. Single particle biophysics approaches have shown the presence of several stable intermediate HA conformations prior to the formation of the irreversible post-fusion state, but they have not been visualized in situ on virion surfaces. We have previously developed a deep learning-based analysis pipeline to improve the throughput of cryoET analysis using convolutional neural networks and quantitively characterize native influenza virions. This approach enabled us to determine a near-nanometer resolution of influenza HA. Leveraging this pipeline to study influenza entry, we have conducted ultrastructural studies of influenza virions incubated with a sialic acid-containing receptor mimic. While receptor binding did not result in significant changes in viral morphology or glycoprotein organization, we observed structural intermediates of HA prior to the irreversible post-fusion state. Quantitative structural characterization of HA fusion intermediates will illuminate the crucial intramolecular conformations that are necessary for optimal viral entry. Understanding HA dynamics and changes in viral conformation upon cellular entry will inform future structure-based drug design, antibody discovery, and innovative vaccine strategies.

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Abstract 2281

A new Micro-C pipeline for chromosomal structure visualization at all resolution levels unravels the placement of chromatin inside our nucleus

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Chromatin is the noodle-like DNA and protein complex inside our cells that contains all our genetic information. Studying the 3-D structure of chromatin is important as it paints a clearer picture on how gene regulation and cell cycle control occur. Chromatin can be thought of as being similar to proteins in that they have a primary, secondary, and tertiary structure which would be the position of nucleosomes on DNA, fibers formed by nucleosome contacts, and long-range looping interactions respectively. A method called Hi-C that maps 3-D chromatin contacts genome wide has been a useful technique in studying contacts in DNA and has furthered our current understanding of chromatin structure. In particular, Hi-C has exceled at revealing structure of chromatin in long-range interactions such as looping. However, Hi-C is unable to reach resolutions necessary for understanding the relationship between the structure of the local chromatin fiber, the secondary structure level, where the underlying genes are located. Recently, a modification of Hi-C called Micro-C has reached unprecedented single-nucleosome resolution while also improving upon long-range chromatin loop detection. This has the ability to improve our understanding of the arrangement of chromatin at all levels. However, a major barrier to the widespread adoption of Micro-C is the lack of available pipelines to analyze Micro-C data. To address this, we have been working to create a pipeline for the in-depth analysis of single-nucleosome resolution data. Our new pipeline effectively takes. fastq files from high throughput sequencing data and converts it into an interpretable heatmap. The changes in this pipeline compared to previous Hi-C pipelines includes a separation of read orientation which allow the pinpointing of nucleosome fiber structure, such as the 10-nm fiber, as our pipeline is affixed to more clearly show short-range interactions. As our pipeline will help in analyzing the primary and especially secondary structure of chromatin specifically the arrangement of nucleosomes in 3-D space, some breakthroughs possible with this pipeline are an enhanced understanding of cancer cells, the proteome, and epigenetics all which greatly affect human health.

Thank you to the NIH and CSU for funding.

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Abstract 2309

Using Support Vector Machine Classification to Predict NKX2-5 DNA Binding Sites

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Edwin Peña-Martínez, Jose Rodriguez-Martinez

Transcription factors (TFs) are sequence-specific DNA-binding proteins essential in regulating gene expression in a tissue-specific manner. Determining TF DNA-binding specificity can help to study gene regulatory networks within cells and how genetic variation can disrupt normal gene expression. Over 93% of disease-associated mutations occur within the non-coding region of the genome, which can serve as TF binding sites. However, more than 90000 single nucleotide polymorphisms (SNPs) have been associated with human diseases or traits, making *in vitro* validation both expensive and extensive work. One method to prioritize mutations for validation is by training Support Vector Machines (SVMs) with *in vitro* data to predict changes in TF-DNA binding affinity. During this project, I implemented a large-scale gapped k-mer (LS-GKM) SVM trained with NKX2-5 ChIP-seq data from Human Embryonic Stem Cells. LS-GKM is a sequence-based predictive model that can be trained to predict regulatory DNA elements that TFs bind. I trained four models using ChIP-seq data from NKX2-5; two from ReMap (30037 peaks) and two from ChIP Atlas (15 308 peaks). All four models had AUROC values above 0.80, as well as satisfactory correlation coefficients, indicating a significant degree of reliability. The models were used to score 488 227 DNase I hypersensitive footprints from fetal heart, and NKX2-5 binding motifs were identified among the highest scores. These scores were consistent through all four models as determined by linear regression analysis. As proof of concept, we used our trained LS-GKM SVM models to score five disease-associated variants affecting NKX2-5 binding. Our predictions were consistent with SNP2TFBS, a PWM-based predictive model. In addition, these predictions were validated through electrophoretic mobility shift assay (EMSA), resulting in differential TF-DNA binding affinity. Given these results, we concluded that LS-GKM SVMs can be implemented to identify and score TF binding sites while also becoming a promising tool for identifying mutations. Future steps include selecting the top 1000 fetal heart footprints to find disease-associated SNPs from the GWAS catalog that alter NKX2-5 binding. Our predictions will be validated through a high-throughput TF-DNA binding assay.

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Abstract 2387

Proteome-wide protein-protein interactions reveal core metabolic processes and virulence mechanisms of bacterial pathogens

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Bacteria are responsible for millions of deaths and antibiotic resistance is of mounting concern. Protein-protein interactions are critical to cellular function; thus, predicting these interactions and providing high resolution structure models of protein complexes on a proteome-wide scale will illuminate key biological processes of pathogenic bacteria that may be druggable targets. We employ a combination of residue-residue coevolution, AlphaFold, and RoseTTAFold2 to systematically screen and model protein-protein interactions between 5 million pairs of proteins across 19 human bacterial pathogens. We identify thousands of protein complexes many of which involve essential genes and/or virulence factors that are paramount to cellular function or modulate bacterial pathogenesis respectively. These predicted interactions offer insights into biological processes which include mechanisms for bacterial gut colonization, outer membrane assembly, DNA repair, and tRNA modifications.

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Abstract 2405

Prognostic Utility of RFX1-Mediated Genes in Thymomas

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Daniel Jacobs, Henry Hoang, Andre Kajdacsy-Balla

Objective: To identify possible relations in survival outcomes in thymoma cases with RFX1 gene expression and its regulatory targets.

Background: Regulatory Factor X1 (RFX1) is an evolutionary conserved transcription factor characterized by a winged-helix DNA binding domain and is implicated in biological processes such as cell proliferation and differentiation. RFX1 's dual repressor and activator properties has been shown to contribute to the dysregulation of stem cell properties in various types of cancer, but it's tumorogenic role in thymomas remain unexplored in the current literature.

Methods: Genes with binding site(s) for RFX1 protein in their transcription starting sites were identified and obtained from the MSig database. KM plots of overall survival in thymoma cases in relation to RFX1 and RFX1-regulated mRNA gene expression levels were generated using TCGA data ($n = 120$ total, "high" versus "low" expression groups determined through the kaplanScan algorithm on the R2 platform). Genes shown to be statistically significant ($p < 0.05$ post false discovery rate correction) were selected for enrichment analysis ($p < 0.01$ for significance) to determine which biological pathways may mediate outcome. For those genes also shown to be differentially expressed in regards to survival, expression levels were quantified and compared between various subtypes of thymomas to identify any RFX1-related differentiation between subtypes.

Results: Out of 248 genes, 16 were differentially expressed in thymoma cases with respect to survival. The genes in which higher expression was correlated with worse outcome included CX3CL1, HDAC9, STMN4, TTC16, DUSP4, DMD, MTTP, C2ORF73, RASGEF1A, and ADM. The genes in which lower expression was correlated with worse outcome included CYTH2, CORO6, RFX1, MSL2, PPP1R32, and ASB7. The 16 genes of interest were significantly enriched in the following biological functions: 'regulation of release of sequestered calcium ion into cytosol', 'cholesterol homeostasis', 'inflammatory response', 'actin cytoskeleton organization', and 'regulation of Ras protein signal transduction'. Statistically significant different gene expressions were observed between thymoma subtypes as well, with expression of various genes being most often differentially expressed in Type C and AB in comparison with Type B1 (9/15 and 8/15 genes showed significantly different expression) and type AB versus Type B2 and B3 (6/15 genes for both).

Conclusions: Our study demonstrated that RFX1-mediated genes are differentially expressed in terms of thymoma survival (alongside RFX1 itself). Enrichment analysis showed dysregulation in biological pathways commonly implicated with tumorigenesis such as Ras protein signaling the inflammatory response, and more novel biological roles such as the release of sequestered calcium, though not as well characterized in thymomas. Additionally, the different RFX1 related gene expression profiles in thymoma subtypes may provide clues regarding their pathogenesis and heterogenous outcomes. Overall, our findings highlight RFX1 as a mediator of thymoma progression and it's potential as a therapeutic target for further wet lab investigation.

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Abstract 2445

Increasing Fluorescent Image Analysis Sensitivity to Characterize ZRS Enhancer Activity: Using Computer Vision Tools to Target the Area for Analysis and Remove Fluorescence Bleed-Through

Jacob Perez, *University of California Berkeley*

Kathryn Ball, Kerby Oberg

The zone of polarizing activity (ZPA) is a cluster of cells at the posterior margin of the developing limb. The ZPA directs anterior-posterior patterning by secreting sonic hedgehog (Shh). The ZPA regulatory sequence (ZRS) is the limb-specific enhancer that regulates and localizes Shh expression. To characterize ZRS activity, we electroporated ZRS-GFP reporter and constitutive RFP control constructs into chicken embryo limbs and took digital images with a fluorescent microscope 48 hours post-transfection. To quantitatively compare ZRS and mutant construct activity, we developed an image processing workflow that measures fluorescence in RFP and GFP images. We monitor transfection efficiency by co-transfecting a constitutive-RFP reporter. To account for transfection variability, ZRS activity (GFP fluorescence) is normalized to transfection efficiency (RFP fluorescence). However, the transfected area varies substantially, including regions outside the enhancer activity domain or outside the limb. Additionally, since RFP is overexpressed with a CMV promoter, red light can bleed through the GFP fluorescent filter skewing fluorescence quantification. We hypothesized that limiting fluorescent measurements to either the ZRS activity domain or the ZPA, compared to the whole limb, and removing RFP bleed-through from GFP images would increase the sensitivity of our analysis. We limited fluorescent measurement to three regions of interest (ROIs): 1) whole limb, 2) ZRS activity domain (posterior half of the limb), and 3) the ZPA (posterior third of the limb, within 500uM of the distal tip) using Python computer vision library tools for thresholding, contours, and hierarchical masking. There was a statistically significant difference between ZRS wildtype and mutant constructs with varying levels of activity when limiting measurement to any of the ROIs, but the degree of certainty increased as the size of the ROI decreased: Limb $p < 0.01$, Posterior $p < 0.001$, ZPA $p < 0.0001$ (independent t-test). After exploring different color space models (RGB, HSV, HSL, etc.), we removed RFP bleed-through by decomposing GFP images into their respective RGB elements and measuring light from the green channel. Removing RFP bleed-through resulted in a mean decrease of 30.5% (+/-1.3%) intensity from the GFP images but did not affect the outcome of the statistical analysis. Limiting fluorescence measurement to the enhancer activity domain increased image analysis sensitivity and may be a useful tool to evaluate enhancer activity.

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Abstract 2482

Drug repurposing: Enol-pyruvyltransferase as a novel target for *Mycobacterium tuberculosis*

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Shivani Singh

Multidrug resistance *Mycobacterium tuberculosis* is a global health concern due to rising number of cases. Pyruvyltransferase is a desirable target as it is involved in the first step of peptidoglycan biosynthesis. This enzyme is widely present in various bacteria, algae and yeast but is conspicuously absent in humans. In mycobacterium, (MurA) UDP-N-acetylglucosamine-3-O-enol-pyruvyltransferase transfers enol pyruvyl from phosphoenolpyruvate (PEP) to UDP-N-acetylglucosamine (UDP-GlcNAc) yielding enolpyruvyl-UDP-N-acetylglucosamine (MurNAc). In this study, structure of MurA was generated through homology. It was validated by, Ramachandran plot and used further in studies for molecular docking with the drug bank library of approved drugs alongside first-line drugs. The residues arginine, histidine and aspartic acid displayed conservation in the catalytic site through a sequence alignment. Many leads bind to arginine present in the binding site and this can be indicative of the presence of the PEP site due to their anionic character. The study found potential drugs that can be repurposed against MurA, a novel target due to its unique characteristic and significance within bacterial cell wall synthesis.

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ASBMB

Abstract 2521

AI-enabled multiscale modeling of SARS-CoV-2 replication transcription complex

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Anda Trifan, Defne Ozgulbas, Alexander Brace, Kyle Hippe, Anima Anandkumar, Sarah Harris, Emad Tajkhorshid, John Stone

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) replication transcription complex (RTC) is a multi-domain protein responsible for replicating and transcribing the viral mRNA inside a human cell. Attacking RTC function with pharmaceutical compounds is a pathway to treating COVID-19. Conventional tools, e.g., cryo-electron microscopy and all-atom molecular dynamics (AAMD), do not provide sufficiently high resolution or timescale to capture important dynamics of this molecular machine. Consequently, we develop an innovative workflow that bridges the gap between these resolutions, using mesoscale fluctuating finite element analysis (FFEA) continuum simulations and a hierarchy of AI-methods that continually learn and infer features for maintaining consistency between AAMD and FFEA simulations. We leverage a multi-site distributed workflow manager to orchestrate AI, FFEA, and AAMD jobs, providing optimal resource utilization across HPC centers. Our study provides unprecedented access to study the SARS-CoV-2 RTC machinery, while providing general capability for AI-enabled multi-resolution simulations at scale.

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Abstract 2526

Everything as code

David Van Valen, *California Institute of Technology*

Biological systems are difficult to study because they consist of tens of thousands of parts, vary in space and time, and their fundamental unit—the cell—displays remarkable variation in its behavior. These challenges have spurred the development of genomics and imaging technologies over the past 30 years that have revolutionized our ability to capture information about biological systems in the form of images. Excitingly, these advances are poised to place the microscope back at the center of the modern biologist's toolkit. Because we can now access temporal, spatial, and "parts list" variation via imaging, images have the potential to be a standard data type for biology. For this vision to become a reality, biology needs a new data infrastructure. Imaging methods are of little use if it is too difficult to convert the resulting data into quantitative, interpretable information. New deep learning methods are proving to be essential to the reliable interpretation of imaging data. These methods differ from conventional algorithms in that they learn how to perform tasks from labeled data; they have demonstrated immense promise, but they are challenging to use in practice. The expansive training data required to power them are sorely lacking, as are easy-to-use software tools for creating and deploying new models. Solving these challenges through open software is a key goal of the Van Valen lab. In this talk, I describe DeepCell, a collection of software tools that meet the data, model, and deployment challenges associated with deep learning. These include tools for distributed labeling of biological imaging data, a collection of modern deep learning architectures tailored for biological-image analysis tasks, and cloud-native software for making deep learning methods accessible to the broader life science community. I discuss how we have used DeepCell to label large-scale imaging datasets to power deep learning methods that achieve human-level performance and enable new experimental designs for imaging-based experiments.

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Abstract 2533

Merger of Protein Dynamics Data and Machine Learning toward Assessing the Effect of Mutations

Ivet Bahar, *University of Pittsburgh*

Recent years have seen a growing number of machine learning (ML)-based approaches that assist in advancing structural biology research, especially in structure prediction. However, it is now established that knowledge of structure provides useful but incomplete insights into the mechanisms of function. The bridge between structure and function is structural dynamics. Progress made in physics-based computational evaluation of structural dynamics, not only structure, can be leveraged if used in conjunction with ML methods. One bottleneck for the use of structural dynamics data in ML methods has been the computing time cost of molecular simulations. However, in recent years, with the introduction of elastic network models, it is possible to efficiently evaluate biomolecular properties dependent on structural dynamics, which permits the use of generated data in machine learning algorithms. Given the importance of protein dynamics, it is not surprising that ML studies that leverage structural dynamics data increasingly gain traction. Recent computational studies show that the merger of ML and structural dynamics theory and methods is mutually beneficial: dynamics data incorporated into ML algorithms increases the accuracy of functional inferences, or ML methods put to use for analyzing full-atomic simulations can help extract information on proteins' kinetics. In both cases, the confluence of ML/DL and structural dynamics data generates knowledge that help close the gap between experimental and computed quantities. A major area of application is the evaluation of the effect of mutations, the pathogenicity of single amino acid variants, as well as the impact of deletions on stability, which are accurately predicted by combining elastic network model predictions, sequence-based evolutionary analyses, and machine learning methods.

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Abstract 2536

AI-predicted protein structures improve deep learning models for bioactivity prediction in drug discovery

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Saulo de Oliveira, Kate Stafford, Chetan Rupakheti, Andreana Rosnik

Structure-based virtual High Throughput Screening (vHTS), i.e., computationally docking compounds to therapeutic protein targets and predicting their activity, is now a routine first step in drug discovery. These methods rely on high-resolution structural representations of protein targets, typically crystal structures. However, many high value therapeutic targets, notably kinases and GPCRs, lack experimental structures. Here, we investigate how state-of-the-art predicted structures can impact and improve AI-based drug discovery in four of Atomwise's computational drug discovery capabilities: molecular docking, pose ranking, active/inactive classification, and bioactivity prediction. We focused on kinases and GPCRs as these classes represent a large fraction of drug targets. Performance of molecular docking and pose ranking is lower when docking against predicted structures compared to experimentally determined structures. We then examined the effect of including over 1300 predicted structures when training DL models for three distinct tasks: global classification of actives/inactives for virtual screening, and kinase- and GPCR-specific bioactivity prediction. We find that models for each of the tasks trained with a combination of predicted and experimentally resolved structures achieve better predictive performance than models trained exclusively on experimentally resolved structures. Furthermore, our retrospective analyses support the use of predicted structures in prospective virtual screening campaigns. Our findings suggest that predicted structures are sufficiently accurate to be routinely included as part of training data for general tasks in AI-based drug discovery, as this leads to better generalization and model performance.

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Abstract 2540

Advancing Data Science Technologies and Analytics in the Areas of STEM

LaTanya Brown-Robertson, *Howard University*

The session will be part of a larger panel of experts within the broad session theme and additional question/answer discussion, with each speaker serving as a panelist. Advancing Data Science Technologies and Analytics in the Areas of STEM -Dr. LaTanya Brown-Robertson's talk will focus on the importance of advancing data science technologies and analytics in the areas of STEM. She will explain best practices on how institutions, departments, and faculty can apply big data and analytics to the disciplines of Biology and Chemistry at an HBCU. The talk will also share resources for teaching and research projects related to data science problem-based exercises. This discussion is best for faculty who wish to infuse data science course curricula and other materials into existing courses and integrate theory, computation, and technology in the undergraduate classroom. The discussion will also provide pointers o navigating the process of developing discipline-based certificates in data science.

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Abstract 2542

Where the Rubber Hits the Road - Applying AI to Drug Discovery Projects

Patrick Walters, *Relay Therapeutics*

Over the past decade, we have seen continued growth in the application of Artificial Intelligence (AI) methods in drug discovery. Developments in deep learning have led to a renaissance in Quantitative Structure-Activity Relationships (QSAR) and de-novo molecule generation. Machine learning (ML) methods, which extract patterns from data and use these patterns to make predictions, are now being applied, to varying degrees, in most drug discovery organizations. ML has impacted drug discovery in numerous areas ranging from target identification to organic synthesis planning. In some disciplines, such as image analysis, ML methods are well established and can be systematically validated. However, in other domains, like automated chemical design (ACD), the contributions of AI can be challenging to separate from those of the end-user. This presentation will highlight emerging areas where AI can contribute to drug discovery programs and open issues for the field.

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Abstract 2608

Comparative evaluation of deep transfer learning with learning-from-scratch for Alzheimer disease MRI images Classification

Anuj Tiwari, *University of Illinois at Chicago*

Sugasini Dhavamani, Tushar Patel, Jagadeesh Ramasamy, Sandra Gesing

Alzheimer disease (AD) is the most common cause of dementia which accounts for 60 to 80% of the aging population. Recent large autopsy studies have shown that half of the individuals with Alzheimer's dementia have Alzheimer's disease brain changes in the pathology along with the causes of dementia. Hence, there is an urgent need to identify early detection of AD. Identification of Alzheimer disease and related dementia (ADRD) in brain Magnetic Resonance Imaging (MRI) images is crucial for Alzheimer's disease and related dementia staging as well as customized treatment planning. However, the precise manual delineation of dementia staging is challenging, tedious, and time-consuming. The emergence of computationally cost-saving and time-efficient deep learning algorithms has significantly impacted reliable early diagnosis of ADRD. In this study, the ResNet-50 method is used to examine the effectiveness of the state-of-the-art transfer learning and training from traditional learning-from-scratch on the Kaggle ADRD dataset. The Kaggle dataset consists of four target variables: Non-Demented, Very Mild Dementia, Mild Dementia, and Moderate Dementia. This dataset contains imbalanced T1-weighted MRI scans with no information of what range of slices that the MRI sequences came from, as well as how many unique brains there are. Because of the imbalance of the target variables, SMOTE resampling is used to balance the class distribution. It has been observed that transfer learning statistically outperforms learning-from-scratch in terms of accuracy in the detection and classification of different stages of ADRD in MRI images. The model performance criteria include the receiver operating characteristic curve (ROC curve), Area under the ROC curve (AUC), F1 score, sensitivity, specificity, precision, recall, and overall accuracy. Successful completion of these studies could lead to a novel approach for the early detection of AD and be helpful for the prevention and treatment of ADRD as well as other neuro-inflammatory diseases. Moreover, it will pave the way for a safe and cost-effective strategy for the prevention of ADRD in the majority of the population.

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Abstract 2623

Extant sequence reconstruction is a new method for evaluating and improving ancestral sequence reconstructions to limit the butterfly effect

Michael Sennett, *Brandeis University*

Douglas Theobald

Ancestral sequence reconstruction (ASR) is a likelihood-based method to infer unknown ancestral sequences using a probabilistic model of evolution and a set of aligned extant sequences. ASR is often employed to understand the evolution of gene or protein properties, but ancestral DNA, RNA, and protein sequences are unavailable because they degrade over time. Unfortunately, our lack of real ancient sequences has resulted in our inability to benchmark or validate ASR with real-world examples, which has led to skepticism toward the accuracy of ASR. Therefore, an open and unanswered question in the ASR field is: How to benchmark and validate resurrected proteins? We addressed this question by employing leave-one-out-cross-validation to reconstruct known extant sequences instead of ancestral sequences, which we call extant sequence reconstruction (ESR). With ESR, we can benchmark reconstructions against ground truth to calculate the accuracy, precision, and bias in reconstructions for different protein families, alignments, and models of evolution. Counter to intuition, we observe that improving the evolutionary model, as judged by model-selection criteria, increases the uncertainty of the single best reconstruction. However, improving the evolutionary model increases the probability of the true sequence and reduces uncertainty in the average sequence. This illustrates the importance of carefully selecting the most appropriate evolutionary model to reduce the overall uncertainty in sequence inferences.

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Abstract 2624

Structural classification of peptide-HLA complexes enables accurate high-throughput modeling

Sagar Gupta, *University of Pennsylvania*

Santrupti Nerli, Sreeja Kutti Kandy, Glenn Mersky, Nikolaos Sgourakis

Major histocompatibility complex class I (MHC-I) proteins play a pivotal role in the adaptive immune system as they display 8–15 residue peptides derived from endogenous proteins on the cell surface for immune surveillance. Despite their extreme polymorphism, Human Leukocyte Antigens (HLA, the human MHC) adopt a structurally conserved fold which defines the peptide-binding groove. Recognition of specific peptide features by T-cell receptors (TCRs) provides the basis of adaptive immune responses, and therefore characterizing the peptide conformational space can lead to a better understanding of antigen immunogenicity. While the peptide termini provide conserved anchoring interactions with the HLA peptide-binding groove, accurate modeling of peptide-HLA (pHLA) complexes has been mired by the inability to capture the central bulge of the peptide. Here, an analysis of high-resolution X-ray crystal structures revealed that diverse peptide conformations arise as a result of local changes in the backbone configuration. Thus, we established an internal-coordinate based system to assess structural similarity between peptide backbones and found that pHLA complexes, encompassing a wide range of allotypes and peptide sequences, present a discrete set of peptide backbone conformations. We combined the knowledge of the representative backbones with a regression model trained on Rosetta energy terms to develop RepPred, a structural modeling approach for HLA-A*02:01 structures. RepPred has a median peptide backbone RMSD of 0.54 and significantly outperformed five state-of-the-art pHLA modeling methods. Thus, we establish that our method produces sub-angstrom models thereby reducing the efforts required to experimentally determine pHLA structures.

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Abstract 2652

An Autonomous Enterprise

Janet George, *Intel*

What is an Autonomous Enterprise in the life sciences industry? An Autonomous Enterprise is an enterprise that is prepared to tap into the power of ML and AI. In other words these enterprises are AI ready and they are powered by AI. They know how to win using AI technologies. In the age of AI which has become an existential threat for enterprises, winning with AI has become a necessity. Delayed adoption increases the risk of the enterprises being left behind their competition and the Industry at large, conceding to business erosion and a slow painful long drawn out eventual acquisition or potential death. Like every major epochs, the Internet age, the social media age, the big data age and the AI age, these genetic markers bring profound technological advances and major seismic shifts in the operational aspects of every business. In this discussion we will explore how pioneers combine, investment choices, strategy, organizational behaviors, and technological adoption as essential ingredients for winning with AI, Advanced Analytics and data on their journey.

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Abstract 2694

Precise Prediction of Calpain Substrate Cleavage Sites Through Delaunay Tessellation-based Structural Analysis

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Huxley Marvit, Zachary Sayyah, D. Andrew Carr

Calpains are a family of calcium-dependent proteases ubiquitous in the cytosol of all cells, the active form of which cleaves a specific class of ligands. Previous literature has focused on predicting both whether a given ligand will be cleaved and the location of the cleavage based on sequence conservation among calpain substrates. These techniques are only somewhat effective: in some cases there is a structural component that is necessary for the ligand to be cleaved which is not captured by sequence-based methods. To improve upon the current sequence based models we have created models using structural information. We utilize standard residue featurization techniques alongside geometric analysis of a protein's carbon-alpha Delauney tessellation for featurization of a residue's structural context. We then trained ML models on the processed residues of approximately 3316 proteins from the CaMPDB and AlphafoldDB databases in order to predict if a residue of a protein will be cleaved by a calpain. The RuleFit classification model leads to an AUC of ~99%, a figure substantially higher than those of previous methods. The explainable nature of the RuleFit model further enabled us to analyze what features were most relevant in predicting cleavage as well as what heuristics exist, providing insights into for functional structural conformation of the calpain ligand targets. We also demonstrate that other standard modeling techniques (such as a Random Forest models or neural networks) perform to a similar degree with the selected underlying attributes, indicating that the featurization techniques applied allow for simple heuristics to determine the cleavage sites of calpain substrates.

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Abstract 2700

Getting vanishingly small crystals to stop vanishing: Imaging and analysis for cutting edge structural methods

Miranda Lynch, *Hauptman-Woodward Medical Research Institute*

Elizabeth Snell, Sarah Bowman

Many of the emergent techniques for protein structure determination – methods using XFELs (X-ray free electron lasers), serial synchrotron crystallography (SSX), and microcrystal electron diffraction (microED) – depend on the use of very small crystal samples of the protein targets. Small crystal sizes are needed for these diffraction-based modalities due to the specific requirements of each method. Typical crystal sizes required are in the submicron- to micron-sized range. Methods for finding and characterizing the difficult-to-observe crystal samples are much needed. For crystals in the range of the wavelengths of visible light, standard brightfield imaging for detecting crystal growth is difficult to use for characterizing the samples. Our work presents imaging methods using nonlinear optical microscopy techniques, coupled with novel analytical approaches for the resulting datastreams, to create new approaches for identifying useable samples for these cutting edge structural techniques. We discuss how multiphoton microscopy can be leveraged to observe submicron crystals, and present novel approaches for image processing that enhance detection of these crystals. We demonstrate our imaging and analysis methods on a variety of gold-standard protein samples for which our group has created tunable crystal growth for generating size ranges of crystals. Our goal in this work is to demonstrate an expanded toolkit of techniques available to unlock a wide range of samples for use in the newest protein structure determination methods.

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Abstract 2735

Pre-emptively Avoiding Drug Resistance: Lessons from viral proteases

Celia Schiffer, *Univ of Massachusetts Chan Medical School*

Drug resistance threatens many critical therapeutics through mutations in the enzyme drug target. Mutations can occur both within the active site of an enzyme and at a site distal from the active site. While the substrate envelope can pre-emptively predict where active site mutations are likely to occur. The molecular mechanisms by which combinations of mutations, especially those distal from the active site, alter drug binding to confer resistance are poorly understood and thus difficult to counteract. From our model system of HIV-1 protease to SARS-CoV-2 Mpro we demonstrate the power of these strategies, where we are combining the substrate envelope with machine learning strategies that incorporate parallel molecular dynamics to uncover the molecular mechanisms of drug resistance. This physics-based strategy of identifies critical features that serve as bellwethers of affinity and can be combined with the substrate envelope to guide inhibitor design that decreased the probability of resistance.

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Abstract 2752

Accelerating antiviral discovery by combining machine learning with structural biology

Alpha Lee, *PostEra*

COVID Moonshot is an international open science consortium aiming to discover oral antiviral against SARS-CoV-2, targeting the main protease. Launched in Feb 2020, Moonshot went from fragment hits to development candidates which are now under preclinical evaluation. In my talk, I will discuss Moonshot's journey, specifically how the combination of machine learning and structural biology has accelerated our design-make-test cycle. I will also discuss our vision for pandemic preparedness, and early results from AI-driven Structure Enabled Antiviral Platform (ASAP). ASAP is a NIH-funded antiviral drug discovery center which builds on COVID Moonshot's approach to target flaviviruses, enteroviruses, and coronaviruses. We are applying machine learning to generate potent chemical matter from crystallographic fragment hits, and leveraging high throughput library synthesis guided by models to rapidly expand on promising hits. Aiming to achieve pandemic preparedness, I will also discuss our approaches to preempting resistance, and how these strategic considerations impact drug-hunting.

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Topic Category DNA Recombination, Structure and Topology

Abstract 147

Variation in the expression TER and TERT genes in *Aspergillus nidulans*

Meklit Yimenu, *Lake Forest College*

Petra Urgacova, Natalie Kamau, Karen Kirk

Telomeres are repetitive and noncoding sequences at chromosome ends that prevent DNA loss during replication. The primary enzyme responsible for telomere replication is telomerase. Telomerase is a ribonucleoprotein with two essential components, TER and TERT. TER is an RNA that contains the template for repetitive telomere synthesis, and TERT is a reverse transcriptase. Assembly of the two components occurs in the cytoplasm in *Saccharomyces cerevisiae* and in the nucleus in mammalian cells. The structure of TER in the filamentous fungus *Aspergillus nidulans* is partially conserved in both *S. cerevisiae* and mammalian cells but whether TER migrates to the cytoplasm or remains in the nucleus during telomerase assembly was unknown. We used the heterokaryon rescue technique in *A. nidulans* hyphae and found that TER remains in the nucleus and, thus, telomerase assembly occurs here. To further investigate this observation, we wanted to explore the expression levels of the TER and TERT genes at various life cycle stages. Nearly ten replications of RT-qPCR were used to determine expression levels in mutinucleate hyphae, asexual conidiospores, and sexual ascospores. Surprisingly, we found that the relative expression levels of TER and TERT were at least two-fold higher in the sexual ascospores compared to hyphae, even though, based on PCR, the telomere lengths were similar. We also looked at expression levels in nkuA and POT1 mutant cells. We used these cells because nkuA and POT1 are involved in nonhomologous end joining and telomere protection, respectively, and because we wanted to test whether these mutations affected expression levels in the different cell types. The relative expression levels of TER and TERT were also higher in the ascospores in these mutant cells. Hence, TER and TERT have higher expression in ascospores than hyphae in both wild-type and mutant *A. nidulans* cells. These results imply not only the upregulation of TER and TERT but possibly longer telomeres in the sexual spores.

We would like to thank Lake Forest College for funding our research.

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Abstract 1213

Basis for the Discrimination of Supercoil Handedness During DNA Cleavage by Human and Bacterial Type II Topoisomerases

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Kevin McCarty, Jo Ann Byl, Frederick Guengerich, Keir Neuman, Neil Osheroff

Humans encode two type II topoisomerases, IIα and IIβ, while most bacteria, including *Escherichia coli* and *Bacillus anthracis*, encode gyrase and topoisomerase IV. The double-stranded DNA cleavage reaction mediated by topoisomerases generates a covalent DNA-enzyme intermediate, known as the cleavage complex. Although type II topoisomerases are essential enzymes, their ability to generate double-stranded DNA breaks makes them intrinsically dangerous to genomic integrity. Because of this potentially lethal property of type II topoisomerases, these enzymes are the targets of a variety of anticancer and antibacterial drugs. All of these drugs (known as topoisomerase poisons) increase the levels of cleavage complexes, which can be lethal when approached by DNA tracking systems. Movement of incoming replication forks or transcription machinery can render the type II enzymes unable to religate the cleaved DNA, leading to genome fragmentation. Given that acutely overwound [positively supercoiled, (+)SC] DNA forms ahead of these DNA tracking systems, it is imperative to understand how supercoil handedness affects type II topoisomerase function. Previous work has demonstrated that DNA supercoil handedness differentially affects levels of DNA cleavage mediated by type II topoisomerases. Human topoisomerases IIα and IIβ and gyrase maintain higher levels of cleavage complexes with negatively-supercoiled [(−)SC] over (+)SC DNA. Conversely, topoisomerase IV maintains similar cleavage levels with both DNA types. To determine whether increased DNA cleavage results from greater cleavage complex stability, we monitored the persistence of (+)SC or (−)SC DNA cleavage in the presence or absence of topoisomerase poisons. In the absence of stabilizing drugs, cleavage complexes rapidly fell apart ($t1/2 < 1.5$ s) and their stability did not vary based on supercoil handedness. In the presence of drugs, human topoisomerase IIα- and bacterial gyrase-mediated DNA cleavage with (−)SC had a longer lifetime than with (+)SC or relaxed DNA. Conversely, human topoisomerase IIβ-mediated persistence of DNA cleavage was shorter than that of topoisomerase IIα and did not vary by supercoil handedness. Topoisomerase IV-mediated persistence of cleavage also did not appear to vary by supercoil handedness, although cleavage decreased more rapidly with relaxed DNA substrates that lacked supercoils. Differences in cleavage complex stability did not reflect rates of enzyme-mediated ligation for all species examined. Rather,

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forward rates of DNA cleavage by human type II topoisomerases and bacterial gyrase were faster with (−)SC than (+)SC DNA. Forward rates of cleavage with topoisomerase IV did not differ by supercoil handedness. These findings suggest that the basis for supercoil discrimination during the DNA cleavage reaction lies at least in part in the differential rates of initial cleavage complex formation.

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Abstract 1243

Mechanisms of CRISPR-mediated immunity and applications beyond editing

Andrew Santiago-Frangos, *Montana State University*

William Henriques, Tanner Wiegand, Colin Gauvin, Murat Buyukyoruk, Kasahun Neselu, Edward Eng, Gabriel Lander, Blake Wiedenheft

Mammals, bacteria, and archaea have domesticated transposases (e.g., RAG1 and Cas1) to form adaptive immune systems. Bacteria and archaea acquire resistance to viruses and plasmids by preferentially integrating fragments of foreign DNA at one end of a CRISPR locus. DNA motifs upstream of the CRISPR (i.e., leader) facilitate integration at the first CRISPR repeat. But how do these upstream DNA motifs act over large distances of 130 bp, or roughly 440 Å, to regulate integration allosterically? Here, we determine the structure of a 560 KDa integration complex that explains how the CRISPR leader DNA recruits Cas (i.e., Cas1-2/3) and non-Cas proteins (i.e., IHF). Cas1-2/3 and IHF cooperate to fold the genome into a successive U-shaped bend and a loop. The genomic U-bend traps foreign DNA against the integrase, whereas the genomic loop positions the leader-repeat junction at the Cas1 active site. The foreign DNA and the CRISPR repeat wrap around opposite faces of Cas2, poised for a Cas1-catalyzed strand-transfer reaction. The post-integration structure suggests that strand-transfer releases tension in the DNA loop. Therefore Cas1-2/3 may harness protein-induced DNA tension to favor the completion of the isoenergetic integration reaction. Cas1-2/3 interacts extensively with the leader and repeat without making sequence-specific contacts, and we demonstrate that protein-mediated folding of DNA drives integration into diverse sequences. These results reveal Cas1-2/3 and IHF strain DNA to enhance integration allosterically and suggest a mechanism for the de novo generation of new CRISPRs. Further, to address an urgent need for inexpensive and rapid detection of viruses, we recently repurposed a CRISPR immune signaling pathway to detect SARS-CoV-2 in patient samples.

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Abstract 1246

Mutagenic ligation of ribonucleotides at the end of DNA replication and repair

Melike Caglayan, *University of Florida*

Base excision repair (BER) prevents the mutagenic and lethal consequences of DNA damage generated by endogenous reactive oxygen species and environmental toxicants. Such DNA lesions are highly abundant and can interfere with DNA replication and transcription or lead to genome instability, events that underlie human diseases. BER requires the coordinated action of repair enzymes and involves the substrate-product channeling, or "passing-the-baton," process that entails the hand-off of DNA intermediates from one biochemical step to the next to ensure the accurate repair. DNA ligase (LIG) I or IIIα catalyzes final ligation step following DNA polymerase (pol) β gap filling at the downstream steps of BER pathway. Yet, the molecular determinants that dictate BER accuracy, particularly in the context of pathway coordination, is unknown. Our studies have revealed that BER responses can lead to mutagenic outcomes. We demonstrated that polβ insertion of 8-oxodGTP leads to the ligase failure, while the nick repair intermediates after the mismatch insertions, with the exception of dGTP:T, cannot be ligated by BER DNA ligases. Furthermore, we showed the low fidelity of LIGI and LIGI-deficiency mutations are both important determinants of unfaithful BER. Also, our studies demonstrated that scaffold protein X-ray cross-complementing protein 1 (XRCC1) increases the processivity of BER reactions and stabilizes the formation of polβ/gap DNA and ligase/nick DNA complexes. Recently, we determined X-ray structures of LIGI/nick DNA complexes with G:T and A:C mismatches and uncovered the ligase strategies that favor or deter ligation of base substitution errors. Our structures revealed that LIGI active site can accommodate G:T mismatch in wobble conformation, while it stays in the LIG1-adenylate intermediate during initial step of ligation reaction in the presence of A:C mismatch at 3′-strand. Our overall findings provide the features of accurate versus mutagenic outcomes at the final BER steps where a multi-protein complex maintain accurate repair. Ribonucleotides can be incorporated by DNA polymerases and the subsequent joining of 3′-OH and 5′-P ends in the phosphodiester backbone at the nick by DNA ligase during DNA replication and repair is critical for maintaining genome stability. Although it has been extensively studied for DNA polymerases across families, the sugar discrimination mechanism of a human DNA ligase at atomic resolution is entirely missing. Here, for the first time, we determine X-ray structure of DNA ligase I (LIG1) in complex with nick DNA containing rG:C at the 3′-end and capture the ligase at the final phosphodiester bond formation step of the ligation reaction involving an adenylate (AMP) release. Moreover, we show mutagenic end joining of the nick DNA substrate with preinserted 3′-rG:C by LIG1 *in vitro*. Our findings reveal an important source of ribonucleotides embedded in genomic DNA, which could come from the failure of LIG1 to discriminate against a ribonucleotide at the 3′-end during nick sealing step of DNA replication and repair.

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Abstract 1283

Coupling of the two enzymatic activities of DNA polymerase beta redefine the DNA base excision repair pathway

Zucai Suo, *Florida State University*

Adarsh Kumar, Andrew Reed, Walter Zahurancik, Sasha Daskalova, Sidney Hecht

Base excision repair (BER) is a major cellular pathway for DNA damage repair. During BER, DNA polymerase β (Polβ) is hypothesized to first perform gap-filling DNA synthesis by its polymerase activity and then cleave a 5′-deoxyribose-5-phosphate (dRP) moiety via its dRP lyase activity. Through gel-electrophoresis and kinetic analysis of partial BER reconstitution, we demonstrated that gap-filling DNA synthesis by the polymerase activity likely occurred after Schiff base formation but before β-elimination, the two chemical reactions catalyzed by the dRP lyase activity. The Schiff base formation and β-elimination intermediates were trapped by sodium borohydride reduction and identified by mass spectrometry and X-ray crystallography. Pre-steady-state kinetic analysis revealed that crosslinked Polβ (i.e. reduced Schiff base) exhibited a 17-fold higher polymerase efficiency than uncrosslinked Polβ. Conventional and time-resolved X-ray crystallography of crosslinked Polβ visualized important intermediates for its dRP lyase and polymerase activities, leading to a modified chemical mechanism for the dRP lyase activity. The observed interlocking enzymatic activities of Polβ allow us to propose an altered mechanism for the BER pathway, at least under the conditions employed. Plausibly, the temporally coordinated activities at the two Polβ active sites may well be the reason why Polβ has both active sites embedded in a single polypeptide chain. This proposed pathway suggests a corrected facet of BER and DNA repair, and may enable alternative chemical strategies for therapeutic intervention, as Polβ dysfunction is a key element common to several disorders.

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Abstract 1356

Cut, Copy, and Paste: Intein-directed invasion of halophile genes

Kallie Poon, *College of Holy Cross*

Megan Yurchick, Lily Nguyen, Kenneth Mills

Inteins are mobile genes that interrupt coding sequences of DNA and are transcribed and translated into self-excising segments of proteins. These inteins contain a homing endonuclease that digests genes lacking an intein. The digestion results in a double-stranded DNA break that is repaired via homologous recombination using an intein-containing allele as a template, thus facilitating a cycle of intein homing. To test the sequence specificity of the homing endonuclease, and the effectiveness of intein homing in an extreme halophile, we observed whether the intein from one organism could facilitate intein homing into the intein-less gene of the same or similar species. The archaeal halophile *Haloferax volcanii* has an intein interrupting its DNA Polymerase B gene. We have created shuttle vectors containing an uninterrupted *H. volcanii* gene, an uninterrupted gene from *Halobacterium salinarum*, and a scrambled gene as a negative control. These plasmid vectors are transformed into *H. volcanii* and we determine whether the plasmid has been invaded via PCR. We have shown that the intein can invade *H. volcanii* sequence and are continuing to determine whether it can invade homologous genes.

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Abstract 1538

Understanding telomere-loop regulation by shelterin protein complex

Joana Pashaj, *University of Wisconsin-Madison*

Bianca Chavez, Ci Ji Lim

Telomeres are protective protein-DNA structures at the ends of eukaryotic chromosomes. These unique nucleoprotein structures are elongated by a specialized enzyme known as telomerase. Unwarranted up-regulation of telomerase is associated with 90% of human cancer cells, suggesting tight regulation of telomere length maintenance has high biomedical importance. The human telomeric DNA sequence is made of thousands of TTAGGG double-stranded repeats that is followed by a 3′ single-stranded tail. Since the telomeric tail and the double-stranded DNA region share the same sequence, the tail can invade the double-stranded region to form a lariat-like telomere-loop (T-loop) structure. This reclusive structure supposedly can prevent untimely access of the telomeric tail. The formation of T-loop is controlled by shelterin, a six-subunit protein complex that is composed of TRF1, TRF2, RAP1, TIN2, TPP1, and POT1. In addition to T-loop regulation, shelterin functions to recruit telomerase to the telomeres and promote telomere extension by telomerase. POT1 is responsible for binding the telomeric tail, while TRF1 or TRF2 are responsible for binding the telomeric double-stranded DNA region. We hypothesize that shelterin protein complexes that consist of POT1 and TRF1 or TRF2 will have the strongest binding affinity to the base of the T-loop, which is the displacement loop (D-loop). To test this hypothesis, we have conducted a series of biochemical binding assays using electrophoretic mobility shift assays (EMSAs) in conjunction with single molecule optical tweezers. We show that shelterin not only binds tightly to telomeric D-loops, but also prefers D-loop binding over linear telomeric DNA. Outcome from the above studies provide a biochemical basis to how shelterin stabilizes T-loop by using its unique DNA-binding properties.

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Abstract 1631

Molecular machines in DNA replication

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Many key molecular machines involved in genome replication – such as the replicative helicase and the DNA polymerase processivity factor PCNA – form toroidal structures to encircle the DNA. Because of their topologically closed nature, these ring-shaped structures need to be cracked open and loaded onto the DNA by specific ATP-driven "loaders." In my presentation, I will show our most recent cryo-EM structural studies and mechanistic understanding of some of these "loaders," including the ORC-Cdc6 that loads the MCM2-7 ring, which is the core component of the replicative helicase the Cdc45-MCM2-7-GINS complex, the RFC complex that loads the PCNA ring, which functions together with the leading strand DNA polymerase epsilon or the lagging strand DNA polymerase delta to synthesize DNA, and the Rad24-RFC complex that loads the 911 clamp ring, which functions in DNA damage checkpoint signaling. I will highlight the power of cryo-EM approach in studying these large and often flexible protein-nucleic acid complexes in the native-like solution conditions. Our studies reveal unexpected insights into the DNA replication mechanism.

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Abstract 1681

Clamp loader processing is important during DNA replication stress

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Peter Chien

The DNA clamp loader is critical to the processivity of the DNA polymerase and coordinating synthesis on the leading and lagging strands. In bacteria the major subunit of the clamp loader, DnaX, has two forms: the essential full-length τ and shorter γ. While these forms are conserved across bacterial species, bacteria have evolved three different mechanisms to create them. *Escherichia coli* creates γ through a ribosomal frameshift. *Thermus thermophilus* does so by transcriptional slippage. In *Caulobacter crescentus* the AAA+ protease ClpXP partially proteolyzes τ to form γ. Though this conservation and independent evolution suggests that DnaX processing is important, the purpose of expressing both τ and γ remains unclear. Here we find a bias against switching from expression of a wild type *dnaX* to a nonprocessable τ-only allele in *Caulobacter*. Despite this bias, cells can adapt to the τ-only allele with little effect on growth or morphology. Using transposon sequencing, we identified a synthetic interaction between the τ-only allele and the deletion of the cell division inhibitor *sidA*. We find that loss of the gene *sidA* in the *τ-only* strain slows growth and increases filamentation. Even in the absence of exogenous DNA damage treatment, the *ΔsidA τ-only* double mutant shows induction of and dependance on *recA*, likely due to a defect in resolution of DNA replication fork stalling. We find that some of the phenotypes of the *ΔsidA τ-only* strain can be complemented by expression of γ but that an overabundance of *τ-only dnaX* is also detrimental. The data presented here suggest that DnaX processing is important during resolution of replication fork stalling events during DNA replication stress.

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Abstract 1684

Exploring the local conformation of individual G4 layers in a G-quadruplex construct using site-specific fluorescent base analogues

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The formation of G-quadruplex (GQ), a non-canonical nucleic acid secondary structure, can inhibit the elevated telomerase activity that is common in most cancers. The global structure and stability of the GQs are usually evaluated by spectroscopic methods and thermal denaturation properties. Most of the biochemical processes involving GQs involve local conformational changes of GQs at the guanine tetrad (G4) level. These local conformational changes are difficult to follow as it is impossible to isolate spectroscopic signals of individual layers of a GQ structure. To overcome this problem, using a synthetic GQ-forming DNA strand, we developed a method in which individual G4 layers in GQs are composed of site-specifically incorporated 6-methylisoxanthopterine (6MI), a Circular Dichroism (CD)-active fluorescent base analogue of guanine. In this study, experiments were performed with human telomeric 22AG sequence (5′-AGGGTTAGGGTTAGGGTTAGGG-3′) where 6MI monomers site-specifically replaced guanines at the positions 3,9,15 and 21. The CD and fluorescence properties of the GQ structures with and without the ligands were characterized under various conditions. Further, thermal denaturation studies showed that the stability of the GQ-ligand complex varies depending on the position of incorporated 6MI probes. The results also showed that the local conformation of individual G4 layers in a GQ due to binding the binding of specific ligand varies with the position of the G4 layer in the GQ structure as well as the chemical charecterstics of the ligands used. This method can be used to understand the details of GQ-protein and GQ-drug interactions at the individual G4 layer that will help design new drugs for treating GQ-related diseases.

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Abstract 1710

Testing the dependence of homology arm length on recombination efficiency in *Chlamydomonas reinhardtii*

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Homology-directed repair of Cas9-induced double-stranded breaks can be used to insert DNA into defined positions in a genome. Homology-directed repair requires that the DNA to be inserted has 5′ and 3′ ends with sequences homologous to the DNA on either side of the double stranded break. However, in the single-celled algae *Chlamydomonas reinhardtii*, the optimal length for the homology arms is unknown. This study tested several methods to generate a homology repair template with 0.25 kb, 0.5 kb or 1 kb arms. We first optimized amplification of our reporter cassette containing fluorescent protein and paromomycin resistance marker from the plasmid pOpt-mRuby-paro (Lauersen, K. et al, Appl. Microbiol. Biotechnol., 2015.). Then we successfully amplified regions of Chlamydomonas genomic DNA on either side of the Cas9-induced double-stranded break and attempted to splice together the fragments with Overlap Extension PCR. Despite repeated iterations, the unusually high GC content of our fragments as well as challenging, reaction-specific conditions contributed to our being unable to isolate a combined DNA product. As an alternative method to join the reporter cassette to the left and right homologous arms we attempted a Gibson assembly. Once we obtain the homology repair templates, we will co-transform them into Chlamydomonas along with Cas9/gRNA RNPs to observe the relative efficiency of reporter cassette insertion as homology arm length varies.

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Abstract 1816

Structures of human CST-polα-primase complex bound to telomere templates

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Telomeres protect mammalian chromosome ends and confer genome stability. An essential step in telomere maintenance is the C-strand fill-in process, which is the de novo synthesis of the complementary strand of the telomeric DNA. This step is performed by polymerase-alpha/primase complex (pol-α/primase) and stimulated by a telomere-binding accessory factor, CTC1-STN1-TEN1 (CST). Using cryogenic-electron microscopy (Cryo-EM) single-particle analysis, we report the structure of the human telomere C-strand fill-in preinitiation complex (PIC). The cryo-EM structure reveals a CST and a pol-α/primase co-bound to a single telomere overhang, poised for de novo RNA primer synthesis. Upon PIC assembly, the pol-α/primase undergoes large conformation changes from its apo-state; CST partitions the DNA and RNA catalytic centers of pol-α/primase into two separate domains and positions the 3′ end of an extended telomere single-stranded DNA template towards the RNA catalytic center (PRIM1 or p49). This structure provides the premise for a physical model explaining how the RNA to DNA synthesis handover is controlled and facilitated by the flexibility for the PRIM2 C-terminal domain. To test this model, we will perform direct enzymatic assays and DNA-binding experiments using recombinant pol-α/primase with a longer or deleted PRIM2 C-terminal domain to increase or decrease, respectively, the flexibility of this domain. The results from these experiments will provide new insights to the molecular mechanism of a CST-pol-α/primase RNA-DNA synthesis handover at the telomeres.

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Abstract 1825

Unparalleled Approaches to Directly Visualize DNA-Binding Proteins and Biomolecular Condensates

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The structure, regulation, and maintenance of DNA is critical to cellular function. Detailed molecular insights into these processes are essential for understanding their associated pathological conditions, establishing and validating biological models, and developing novel therapeutics. As a result, direct, real-time observations of individual proteins interacting with DNA are needed to obtain a complete picture and to validate current biological models. Technologies, such as the C-Trap, offer an exciting opportunity to meet these challenges by enabling researchers to observe DNA processes in real-time at the single-molecule level. Biomolecular condensates are also critical to cellular function. For instance, phase separation of proteins and RNA can cause formation of membrane-less organelles – e.g. stress granules, RNA-transport granules – that allow for efficient interactions between those biomolecules. There is increasing evidence that membrane-less condensates are implicated in human diseases such as cancer, amyotrophic lateral sclerosis, and Alzheimer's disease. Understanding the formation, physical properties, and mechano-chemical interactions of membrane-less organelles will provide essential information about their molecular basis and associated pathologies. Here, we present our efforts to enable unparalleled molecular-level discoveries using a combination of optical tweezers with fluorescence microscopy. We present examples in which our technologies enhanced the understanding of DNA regulators, DNA repair mechanisms, and DNA editing tools (e.g. CRISPR/Cas) in previously unattainable ways. We also show novel assays to investigate the properties and behavior of biomolecular condensates. Importantly, we show that advances in single-molecule technologies can be turned into easy-to-use instruments that enable discoveries previously reserved for the world's most specialized biophysics labs.

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Abstract 1829

The *E. coli* Replication Risk Sequence (RRS)

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Michael Cox, Elizabeth Wood, Phoung Pham, Myron Goodman

In the *E. coli* genome are two 222 bp repeats that we have designated replication risk sequences or RRS. These sequences are symmetrically arranged around and 650 kbp away from the dif sequence that marks replication termination. The RRS are GC-rich and capable of forming extensive secondary structure when single-stranded. The RRS trigger frequent deposition of RecA protein on the lagging strand in gaps formed nearby. The RRS play a key role in replication and/or nucleoid structure and segregation. Deletion of one RRS generates a growth defect. We have been unable to construct a strain lacking both RRS. This poster will explore the effects of RRS on replication and transcription in the genome, as well as its effects on plasmid stability when cloned. The RRS are widely conserved in enterobacteria.

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Abstract 1862

Examining the Participation of Candidate Genes in Bdelloid Rotifer DNA Repair

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Bdelloid rotifers are microinvertebrates found in aquatic environments around the world. The bdelloid *Adineta vaga* can recover from extensive DNA damage caused by desiccation due to an incredible DNA repair system. However, it is presently unknown how this DNA repair mechanism works. When exposed to ionizing radiation, *A. vaga* experiences DNA double strand breaks in its genome. Previous work identified several upregulated genes in irradiated bdelloids, including DNA polymerase beta (POLB) and a DNA ligase homolog (that we designated bdLIG). This project aims to uncover the origin and function of these two upregulated genes in bdelloid rotifers. Two copies of POLB are present in the *A. vaga* genome. A phylogenetic analysis showed *A. vaga* POLB was present in a clade containing other DNA polymerases (including β, γ and μ). We identified four copies of bdLIG in the *A. vaga* genome. Our phylogenetic analysis revealed that bdLIG is within a lineage comprised of homologs from bacteria and diverse eukaryotes that is distinct from DNA ligases 1, 3, and 4, which are universal to most eukaryotes, including bdelloid rotifers. The domain structure of bdLIG proteins was also distinct from DNA ligases 1, 3, and 4. To investigate the function of POLB and bdLIG, we used CRISPR to inactivate the genes. First, we designed and amplified single guide RNAs (sgRNAs) to target POLB and bdLIG. We verified that the sgRNA/Cas9 complexes cut each target gene using an *in vitro* cleavage assay. Single-stranded oligodeoxynucleotide (ssODN) templates were designed for POLB and bdLIG to insert mutations into each target gene via homology-directed repair. To perform CRISPR for each gene, embryos were electroporated with the sgRNA/Cas9 complex and ssODN. We used PCR and deep amplicon sequencing to screen embryos for mutations. Once identified, mutants will be tested and assessed on their ability to recover from desiccation to ultimately uncover if the DNA repair process is affected by the inactivation of these genes. This research is important because it will broaden our understanding of DNA repair beyond traditional model organisms.

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Abstract 1979

A DNA Binding Protein Recruits Type II Topoisomerases by Direct Interaction to Promote DNA Replication

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DNA replication is a necessary and broadly conserved process for all life. A significant obstacle to DNA replication is positive supercoiling, DNA strands wound about themselves from the strain of a progressing replication fork. Type II topoisomerases (Top2s) are essential and ubiquitous DNA replication enzymes that relax positive supercoils to enable replication. Despite decades of study, it is not known if the intrinsic affinity of Top2s to positive supercoils is sufficient for the relaxation of supercoiling. We recently discovered that Growth-Associated Protein in Regulation (GapR), an essential DNA binding protein in alphaproteobacteria, binds positive supercoils specifically and stimulates the activity of Top2s DNA Gyrase and Topoisomerase IV during DNA replication. However, the mechanism for GapR stimulation of Top2s was not known. To interrogate the interaction, we used direct interaction assays including pull-down with 6xHis-tagged GapR, 3xFLAG Immunoprecipitation with Top2 subunits, and the Bacterial Two-Hybrid assay. In these assays we detected a specific interaction between GapR and Top2s: 6xHis pull-down and 3xFLAG IP identified corresponding interaction targets, and Bacterial Two-Hybrid identified a direct interaction between the GyrA and ParC Top2 subunits. Next, we aim to identify the surface that mediates direct interaction between GapR and Top2s, revealing a previously unknown mechanism of Top2 recruitment. We hypothesize that disrupting the GapR-Top2 interaction will lead to cell death, and as GapR is broadly conserved by alphaproteobacteria, our research could reveal a novel mechanism to inhibit with antibiotics. If a conserved mechanism, our work could identify new anticancer therapeutics, as human Top2 inhibitors are important chemotherapy drugs.

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Abstract 2034

Cre-lox Recombination Engineering Guided by Machine Learning for Comprehensive Analysis of Neural Networks

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Background: Neural networks are responsible for generating complex behavior. Their relationships are, however, poorly understood. Thus, we developed "functional cellomics," which allows for the hypothesis-free functional annotation of neural networks. The critical point is the stochastic labeling of opsins by the stochastic recombination by Cre. We designed a genetic cassette named pSTAR containing two sets of lox variants, lox2272 and loxP, in an alternate way. The transcription factor QF2w is inserted between loxP. Cre excites DNA with an exclusive choice of lox2272 or loxP. Expression of QF2w induces opsin only when Cre excises lox2272. We successfully implemented the stochastic labeling of opsin dependent on Cre induction by constructing a *C. elegans* strain carrying the pSTAR gene cassette [1]. However, the opsin labeling rate was about 30%. By labeling a large subset of neurons by opsin, it becomes difficult to distinguish the function of each neuron. In this study, we demonstrated a strategy for precisely controlling the labeling rate of opsin or any other effectors by machine learning-guided engineering of Cre-lox recombination. Method Neurons that experience Cre-lox recombination between lox2272 sequences express the opsin coding gene. Therefore, we hypothesized that the opsin labeling rate would be reduced using lox2272 variants, which are difficult to be excised by Cre. Thus, we generated a library of lox2272 sequences by introducing mutations using PCR and introduced them into *S. cerevisiae*. Then we induced Cre and extracted DNA from *S. cerevisiae*. We used Illumina sequencing to evaluate the excision rate of the lox2272 variants. To develop a machine learning model that predicts the excision rate of lox2272 variants, we trained a Gaussian process (GP) model using data from Illumina sequencing containing lox2272 sequences and their excision rates. First, the dataset of about 1000 variants was divided into a training dataset and a test dataset at a ratio of 9 : 1. Next, we built a GP model using the training dataset. We verified the prediction accuracy of the test dataset. Using the GP model, we predicted the cleavage rate of unevaluated lox2272 sequences and verified them by qPCR. Results We acquired the cleavage rate of more than 2000 mutant lox2272 sequences. We successfully found lox2272 variants with excision efficiencies ranging from 0.05% to 100%. The results of the Illumina sequencing analysis were confirmed by qPCR. Next, we built a GP model and observed a high correlation between the actual and predicted cleavage rates of the test dataset (R = 0.93). Furthermore, the correlation between the measured cleavage rate of the unevaluated lox2272 sequence and the predicted cleavage rate was also very high (R = 0.96). Using the GP model, we succeeded in discovering lox2272 variants that can achieve a cleavage rate in the range of 5.7×10^{-4}% – 41% [2]. Conclusions In this study, we constructed a GP model that can predict the cleavage rate of lox2272 variants. The mutant lox2272 sequence with a cleavage rate of 5.7×10^{-4}% enables the sparsest labeling among the previous studies [3–5].

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Abstract 2250

Modifications of the LacZ gene in *E. coli* through CRISPR

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Lactose intolerance is a common digestive issue, usually caused by a deficiency of the β-galactosidase enzyme, otherwise known as lactase. The lactase enzyme is found in the bacterium *Escherichia coli* (*E. coli*) located in the intestines of humans. *E. coli* tunes its metabolism to the environment in which it grows. Therefore, if the habitat is rich in lactose like an infant's intestines, then it will metabolize lactose. However, if in adults, where you can find lactose intolerance, there is less lactose, the *E. coli* would metabolize other molecules instead. With the use of CRISPR, it is possible to turn on the ability to metabolize lactose no matter what environment *E. coli* is formed in. The Independent School BioClub SMART Team used 3-D modeling and printing technology to examine bacterial conjugations in the DH5α strain activating the LacZ gene and therefore the lactase enzyme. Lactose intolerance is typically harmless, but it can cause severe digestive problems if ignored, as it commonly is. Advancements in research of ways to safely trans-conjugate DNA of *E. coli* could potentially lead to an elimination of lactose intolerance.

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Abstract 2407

Cis- and trans-acting factors affecting the location and frequency of de novo telomere addition in yeast

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DNA double-strand breaks threaten genome stability since incorrect repair may lead to chromosome rearrangements accompanied by DNA loss or gain. Some sequences are at elevated risk for breakage and/or incorrect repair, raising questions about how selective pressures act on such sequences to shape genome structure and function. We are addressing these questions through studies of chromosome regions that are hotspots of new (de novo) telomere addition (dnTA). Telomeres are repetitive sequences that protect linear, eukaryotic chromosomes from degradation and facilitate complete replication by recruitment of the enzyme telomerase. In contrast to the stabilizing role of telomeric sequences at chromosome ends, interstitial telomere-like sequences can promote sequence loss if acted upon by telomerase to generate a new telomere. In humans, dnTA at genomic "hotspots" is observed in multiple diseases. We seek to understand why certain sequences trigger dnTA at increased frequency and how such sequences persist despite their apparently negative consequences. We are addressing these questions through analysis of sequences in the budding yeast (*Saccharomyces cerevisiae*) genome that undergo dnTA at elevated frequency (Sites of Repair-associated Telomere Addition; SiRTAs). In prior work, we characterized cis-acting sequences required to stimulate dnTA and uncovered a critical role for the single-stranded telomere binding protein Cdc13. However, a description of SiRTA distribution and function on a genome-wide scale is lacking. To address this deficit, we developed a computational algorithm to predict SiRTA function based on similarity with the TG1-3 pattern of the yeast telomeric repeat. In parallel, we have validated a high-throughput sequencing method that dramatically increases the number of potential SiRTAs that can be characterized. The algorithm predicts SiRTA function with high accuracy (false positive and negative rates of less than 5%) and, for sequences predicted to stimulate dnTA, is predictive of the level of dnTA ($r^2 = 0.57$). Using the algorithm, we identify TG-dinucleotide repeats as some of the strongest sites of dnTA in the genome. While there is no apparent bias in SiRTA location across most of the genome, all but one of the subtelomeric repetitive regions (X and Y' elements) contain at least one functional SiRTA that may facilitate telomere healing in the event of catastrophic telomere loss. In parallel with efforts to define the cis-acting sequences that contribute to SiRTA function, we are using biochemical and genetic approaches to define proteins that regulate this process. Using competitive fluorescence anisotropy binding assays, we find that SiRTA function is correlated with Cdc13 binding, although binding affinity alone is insufficient to explain

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all variation in the frequency of dnTA. In a genetic screen, we identified multiple genes that impact dnTA at SiRTAs, including genes needed for telomerase function and DNA double-strand break processing. Loss of Ubp10, a ubiquitin protease with roles in error-prone repair and maintenance of telomeric chromatin, affects repair pathway choice at a SiRTA, causing a shift from repair by telomere addition to translocation, likely through break-induced replication. Together, this work provides a foundation for developing a fuller understanding of how sites with a propensity to stimulate dnTA impact genomic instability and evolution.

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Abstract 2420

The effects of structural changes in PALB2 (partner and localizer of BRCA2) ability to bind DNA

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DNA is susceptible to damage by UV light, radiation, or even DNA replication, which can cause breaks in the genome, eventually leading to cell death or cancer. However, these breaks are normally corrected by replacing the damaged or missing DNA fragment with an undamaged copy through DNA recombination, a process commonly known as homologous recombination (HR). HR requires a plethora of proteins that recruit a major recombinase RAD51 to sites of DNA damage. An important protein in this process is PALB2 (partner and localizer of BRCA2) which coordinates the activities of several proteins, including BRCA1 and BCRA2, important for RAD51 activation. Therefore, malfunctioning of PALB2 can increase cancer risk in tissues such as the breasts and ovaries. Our lab recently discovered that PALB2 itself can recombine DNA. Moreover, the DNA-binding domain (DBD) of PALB2 which carries this activity is unstructured, except one alpha-helix at the N-terminus, which stimulates homodimerization. We demonstrated that temperature denaturing of PALB2-DBD did not affect its DNA-binding activity as typically observed for other intrinsically disordered proteins (IDPs). Our study aimed at understanding the effects of different structural changes in PALB2 on its DNA binding and strand exchange abilities. These changes are probed by fluorescent methods through the incorporation of fluorescent labels to the protein through thiol coupling reaction using available or newly introduced cysteine amino acids. Since any sequence alteration can affect protein function, our main goal was to validate all cysteine mutants and the protein labeled with fluorophores for the DNA binding activity. We used a recombinant version of PALB2 protein, PALB2-DBD consisting of the first 195 amino acids of PALB2 (the unstructured region that binds to DNA) for these assays. Validated protein constructs are being used in structural studies using different biophysical approaches to further enhance our understanding PALB2 function.

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Abstract 2446

Circularization of Chromosome XV in *Saccharomyces cerevisiae*

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Prokaryotes have circular chromosomes while eukaryotes have linear chromosomes with ends called telomeres. While the evolution of linear chromosomes in eukaryotes is thought to permit meiosis and sexual reproduction, telomeres have an evolutionarily unfavorable condition known as the end-replication problem, where the lagging strand of DNA is not fully copied which, given multiple rounds of replication, would result in the loss of genetic information. To counteract the end-replication problem an enzyme complex called telomerase evolved to add nucleotide repeats to the end of the chromosome to prevent the loss of genetic information. In an effort to better understand the evolution and function of linear chromosome architecture, we are genetically engineering a simple eukaryotic organism, *Saccharomyces cerevisiae*, with circularized versions of their linear chromosomes. Our approach utilizes two DNA cassettes with selectable marker genes inserted into the ends of a single chromosome. The DNA cassettes have homologous regions that can induce DNA recombination to join the ends of the chromosome together, eliminating the telomeres and creating a single circular chromosome. Using this approach, I have successfully circularized chromosome XV. Initial phenotypic characterization shows the circular chromosome has no defects relative to the linear version, suggesting eukaryotes can survive without telomeres. Ultimately, these experiments will allow us to better understand the function and evolution of linear chromosomes and telomeres in eukaryotes.

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Abstract 2566

The MRN complex and regulation of recombination in eukaryotic cells

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DNA double-strand breaks are recognized, processed, and signaled by the Mre11-Rad50-Nbs1(Xrs2) complex in eukaryotes. The MRN complex has a unique structure that includes the Mre11 nuclease, the Rad50 ATPase with long coiled-coil arms resembling the SMC family of proteins that controls chromosome topology, and the Nbs1 regulatory protein that controls the enzymatic components of the complex. MRN binds specifically to double-strand break ends and holds these in a partially unwound configuration that promotes both processing and recognition by the ATM protein kinase that is essential for checkpoint responses to double-strand breaks. The MRN complex and ATM are associated with repair of DNA double-strand breaks through homologous recombination, which requires resection of 5′ strands to form 3′ single-stranded DNA intermediates. Mre11 plays a key role in the resection process by initiating the 5′ resection with its endonuclease activity, promoted by phosphorylated CtIP. However, in mammalian cells, most double-strand breaks are repaired through non-homologous end joining (NHEJ). The relationship between NHEJ and homologous recombination has been modeled as a competition between MRN and the core NHEJ factors ("pathway choice"). Here we address several intersections between MRN and the non-homologous end joining machinery, showing how MRN and ATM functions can be regulated by NHEJ factors and also how MRN and NHEJ proteins affect DNA end processing in mammalian cells. These findings indicate that NHEJ and homologous recombination share common stimulatory factors and operate in a sequential rather than competitive relationship in human cells.

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Abstract 2615

Cryo-EM investigation of a single strand annealing homologous DNA recombination protein, ICP8 annealase from HSV-1

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Timothy Newing, Jordan Nicholls, Adile Kaban, Bhanu Mantri, Gokhan Tolun

Human Herpes Simplex Virus-1 (HSV-1) is a double stranded DNA virus, that is estimated to be carried as a latent infection by two thirds of the world's population. In many people, HSV-1 periodically reactivates giving rise to a lytic infection that manifests as mild cold sores around the mouth and nose. However, in extreme cases or in immunocompromised individuals, HSV-1 infection can increase the risk of HIV infection, the development of certain cancers or result in severe complications such as meningitis and encephalitis. Antivirals are available for the treatment of infection; however, resistance is on the rise. Single Strand Annealing DNA recombination (SSA) is a pathway involved in the replication and repair of DNA, and it is conserved in all tiers of life from bacteriophages to humans. SSA is catalysed by an Exonuclease Annealase Two-component Recombinase (EATR) protein complex. The exonuclease binds to double-stranded DNA ends, digesting one strand in the 5′ to 3′ direction exposing a single stranded DNA overhang. The annealase binds to this single stranded DNA and catalyses homology searching and subsequent annealing between homologous regions of two single stranded DNAs. HSV-1 utilises an EATR comprised of the annealase Infected Cell Protein 8 (ICP8) and the exonuclease UL12. In addition, both proteins have numerous other roles in infection, viral DNA replication, and are essential for the production of infectious viral progeny. Therefore, the two proteins represent attractive antiviral drug targets. To inform structure-based drug design and provide a greater insight into the molecular mechanism of SSA, this project aims to use cryo-electron microscopy to determine 3D structures of ICP8 and UL12 both individually and as an EATR complex. The proteins were expressed in SF9 insect cells and purified by affinity chromatography, with cryo-EM performed on an FEI Talos Arctica electron microscope. ICP8 is known to form double-stacked nonameric rings and bipolar filament superstructures that are functionally important. In this study, ring structures were formed by incubating ICP8 sequentially with two complimentary 105mer oligonucleotides. FRET gel mobility shift assays indicated an optimal protein:DNA ratio of twice the stoichiometric binding capacity of ICP8. Negative-staining EM and mass photometry were also used for further optimising protein-DNA complex formation. The resulting ring structures were crosslinked with glutaraldehyde and purified by gel-filtration chromatography prior to preparation for cryo-EM. Bipolar ICP8 filaments were formed by prolonged incubation of ICP8 both in the presence and absence of DNA. We have used cryo-EM to reconstruct preliminary 3D volumes of ICP8 in both the ring and helical forms, into which an existing crystal structure of the N-terminal domain of ICP8 has been fitted. By comparing the models of these two oligomeric forms, we can begin to understand the role of oligomerisation in SSA for HSV-1. This work provides a foundation for ongoing efforts to obtain higher resolution structures, which will provide insights into the mechanisms underlying SSA.

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Topic Category Education and Professional Development

Abstract 146

Diversity is the Key to Innovation-Connecting diversity in the Molecular Foundry user program and beyond

Phinn Markson, *Edgewood College*

Shannon Ciston

This report investigates and evaluates diversity, inclusion, and equity (DEI) among users at Berkeley Laboratory's Molecular Foundry. The researchers assessed and quantified inclusion among users from Minority-Serving Institutions (MSIs) in the user programs and proposal review boards by compiling demographic data of Foundry users, analyzing trends over time, and testing correlations between user proposal acceptance rates from MSIs and primarily white institutions (PWIs). The results show a mean average of a nine percent difference in user proposal acceptance rates from MSIs and PWIs from 2015 to 2021. In addition, demographic trends over time demonstrate a lack of growth specifically among Black, Latinx, and Native American users. The discrepancy in user proposal acceptance rates from MSIs and the lack of increase among underrepresented minorities highlight the need for formal and strategic outreach programming. The research group recommends that the Foundry develop and implement DEI outreach programming by partnering with diverse institutions, engaging in existing programs, establishing mentoring programs, and creating a more robust visiting faculty program.

This work was supported by the U.S Department of Energy, Office of Science, Office of Workforce Development for Teachers and Scientists (WDTS) under the Community College Internship (CCI) program.

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Abstract 156

The Biochemistry Authentic Scientific Inquiry Lab (BASIL) provides a framework for learning Michaelis-Menten kinetics

Rebecca Roberts, *Ursinus College*

BASIL (Biochemistry Authentic Scientific Inquiry Lab) is a course-based undergraduate research experience (CURE) that focuses on enzyme function prediction. The Protein Data Bank (PDB) contains the structures of over 4000 proteins of unknown function and BASIL provides a basis to assign functions by combining computational and *in vitro* analyses. The final BASIL module focuses on a kinetic analysis of the purified protein. In this pilot study we investigated if the BASIL CURE promotes the development of student understanding of Michaelis-Menten kinetics. Through content analysis of the written BASIL modules and inductive analysis of instructor interview transcripts, an "expert answer" was determined and categorized into three overarching themes: the function of an enzyme is to convert a substrate to a product; an assay can visualize the conversion of a substrate to a product; and data from an assay needs to be graphically organized to yield useful information to characterize function. The ideal answer subthemes then were aligned with the course-based undergraduate research abilities (CURAs) identified by Irby et al. (2018). Student research posters and semi-structured interviews with students who have participated in BASIL at Ursinus College were analyzed in order to compare them with the "expert answer." Results suggest that students appear to understand the function of an enzyme and can interpret findings from an activity assay, yet they are lacking an in-depth understanding of the required data manipulation or underlying meaning of a graphical analysis of kinetics data. In the longer term, this research is aimed at informing improvements in the teaching, learning, and assessment of this area of the BASIL CURE. In particular, the findings will focus on addressing the various identified student difficulties so that the BASIL CURE can come closer to achieving its primary goal of developing students as research scientists.

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ASBMB

Abstract 162

Perceptions of e-Learning by Deaf and Hard of Hearing Students Using Asynchronous Multimedia STEM Tutorials

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Lisa Elliot

COVID-19 has impacted education in innumerable ways at all levels. For institutions of higher education, budgetary deficits, fluctuating enrollments and personnel decisions have come to the fore, galvanizing efforts to expand education to diverse student audiences, including online curricular portfolios. Developing digital materials for learning purposes, or e-learning materials, is not as straightforward as it may seem. The traditional approach is multimedia instruction, the presentation of both pictures and words in an e-learning format to foster learning. However, multimedia instruction, to develop meaningful learning, needs to support the active processing of the learner who engages with those materials. The Cognitive Theory of Multimedia Learning (CTML) informs the development of effective multimedia e-learning materials. However, given the dearth of CTML studies directed at students of diverse and underrepresented populations, such as d/Deaf and hard-of-hearing students (DHH), it is unclear as to the extent that CTML principles may inform effective multimedia instruction for this group. We recruited DHH students to view an asynchronous, online multimedia tutorial covering foundational chemistry concepts applicable to biochemistry and to complete a brief questionnaire that inquired about their e-learning perspectives. A mixed-methods approach was used to characterize responses. Students characterized their use of the tutorial's features as broadly serving three different functions and also placed a strong emphasis on the benefits of the accessibility features. These findings have implications for the applicability of at least one CTML principle for this student population. At the same time, DHH students both perceived learning benefits from tutorial features and suggested additional improvements to the tutorial that invoke other CTML principles. The survey and recruitment procedure were reviewed and approved by the RIT Human Subjects Research Office (Federal Wide Assurance# FWA 00000731). Informed consent was obtained from all individual participants included in the study.

This study was funded by a Ronald D. Dodge Memorial Endowment Fund grant.

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Abstract 1176

Diversifying academia: Understanding and implementing equitable and inclusive hiring practices through faculty learning communities

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Erik Arevalo, Eva Fuentes-Lopez, Mike Wilton

Academia has historically been a space where majoritized individuals have been represented. More recently, student demographics are becoming more diverse; however, faculty demographics have not followed suit at the same rate. This presents a critical issue as the lack of representation within faculty limits not only the success of students from diverse communities but also the diversity of ideas in academia. To address this issue, we utilize the context of search committees to examine how equitable and inclusive hiring practices are understood and implemented by faculty in science, technology, engineering, and mathematics (STEM) disciplines at four research-intensive institutions. Using an iterative design-based research approach, a total of about 10 faculty on search committees from the four campuses each year engage in faculty learning communities, peer-led structured groups that support collaborative discussion and reflections. Specifically, for the first two years of the project, we examined research literature on how to write the job advertisement and discussed the potential barriers and support in different departmental contexts for implementation. We identified four dimensions relating to equity and inclusion in the advertisement: use of language to describe the ideal candidate; description and placement of the institutional commitment to diversity, equity, and inclusion (DEI); biases and assumptions built into the evaluation criteria; and promotion and dissemination of the advertisement. In terms of barriers and support for implementation, we found that the job advertisement can be constrained by institutional structures on what is allowed to be included. However, the values and visions of a department are symbols that can be reflected in the language choice, placement and description of a DEI statement, and the inclusivity of how requirements of the position are evaluated. Individuals on a search committee are people with goals, agency, needs, and identities that may be embedded into the advertisement, and they also have strategies for distributing the advertisement that are reflective of their professional networks. We also found that the writing of the advertisement is influenced by power, i.e. the status, positioning, and political coalitions of members of the committee, department, and institution. While structures can be represented in the form of policies and symbols can guide how the structures are spoken about, power ultimately determines how specific implementations are enforced. Through this work, we are able to make key recommendations for all aspects of the hiring process that could support the diversification, inclusion, and belonging of minority faculty

from minioritized communities across STEM disciplines at different institutions.

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Abstract 1177

Faculty conceptions of diversity, assessment, grading, and student ability are linked to racial disparities in course grades

Stanley Lo, *University of California San Diego*

Nicole Suarez, Elizabeth Park, Mike Wilton, Song Wang, Stacey Brydges, Natascha Buswell, Brian Sato

Institutions have increasingly made the commitment to diversify higher education, yet racial disparities in science, technology, engineering, and mathematics (STEM) disciplines continue to persist. Faculty interact directly with students and have important roles in creating an inclusive classroom culture and thus reducing opportunity gaps for student achievements that result in such racial disparities. Our study asks two research questions: How do faculty conceptualize diversity in higher education, and how do related beliefs about assessment, grading, and student ability contribute to racial disparities and opportunity gaps? First, we examined the qualitatively different ways in which faculty experience and understand diversity. Data were collected through semi-structured interviews with 39 faculty from two-year and four-year minority-serving institutions. Codes emerging from the data were discussed by four researchers with diverse positionalities to support validity, and transcripts were coded by two independent researchers to ensure reliability. Variations among participant experiences were organized into three distinct conceptions of diversity. In Conception I (which we termed essentialist), faculty attend to demographic features of students and view students with a fixed mindset of ability. In Conception II (functionalist), faculty attend to different student viewpoints and consider students with a deficit mindset. Conception III (existentialist) attends to how lived experiences shape the kinds of learners that individual students become in the classroom. Implicit power dynamics are considered, and students are viewed as rightfully present in higher education. To address the second research question, 216 STEM faculty were surveyed to assess their beliefs about diversity, assessment, grading, and student ability. Survey results were linked to 31361 unique student transcript data spanning three academic years. The results show that faculty with fixed ability beliefs endorse more traditional mindsets regarding diversity, assessment, and grading such as adopting a colorblind ideology about diversity, considering the purpose of course assessment as sorting students instead of providing feedback, and using grading as a gatekeeping mechanism. The endorsement of these traditional beliefs is also associated with larger opportunity gaps in course grades, suggesting that faculty beliefs may be driving racial disparities in STEM disciplines. Furthermore, analysis of faculty characteristics indicates that male faculty, full professors, and instructors in physical sciences tend to hold instructional beliefs that are linked to opportunity gaps that result in racial disparities in course grades. Overall, our results indicate that while faculty acknowledge different student features and have varying understanding for what diversity

means in higher education, some conceptions of diversity, assessment, grading, and student ability do not necessarily suggest an inclusive culture.

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Abstract 1178

R@N: an Asynchronous, Student-Led Program to Train Students to Conduct Independent Research

Houjun Liu, *The Nueva School*

Michael Sarboraria, Vinca Lu, Kian Salimi, Oliver Cho, Flint Kuznetsov, Zachary Sayyah, Albert Huang, Paul Hauser

Most high-school science programs have a strong focus on scientific theory and do not train students to conduct independent research. Previous work has demonstrated the efficacy of a mentor-supported, student-driven teaching program to effectively introduce research-specific skills in a classroom context. Despite the effectiveness of such programs, their class-based formats and requirements for multiple full-time faculty mentors limit their throughput, and the finite expertise of full-time mentors requires participants to focus on specific research subjects. To address these limitations, we introduce R@N, an extracurricular, student-led, and student-driven program for the independent acquisition of research-specific skills through the self-guided completion of a series of formative checkpoints ("nodes") for mastery. Students in the program can choose specific subsets of nodes to be trained in research in subjects of their interest. The program is developed and moderated by a small team of students in consultation with skill-specific faculty mentors through regular meetings. Students meet weekly to create, update, and revise nodes in collaboration with mentors in order to enable and supplement the learning of students participating in the program. The program offers a few key results: it electively allows the student body (approximately 400 in our institution) to asynchronously acquire the skills of independent research and enables a group of around 20 students to develop and codify tools and skills for research pedagogy. The program can be sustained with limited faculty involvement, requiring one dedicated faculty mentor working in conjunction with a larger pool of research mentors who commit around 2 hours per month.

The program is supported by The Nueva School.

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Abstract 1194

Project80 3.0: An Updated Student-Driven Framework for Creating Multimedia Educational Resources from Primary Literature

Anoushka Krishnan, *The Nueva School*

Micah Brown, Houjun Liu, Grace Holmes, Paul Hauser, Luke De

Conventional high-school pedagogy focuses primarily on teaching codified theory, and doesn't place an emphasis on current scientific discourse. Project80 is a student-run team that digests high-level current scientific discourse to create nuanced, accessible podcasts for the high-school audience. Last year, we tested the efficacy of two protocols for podcast creation. We first produced an episode via a protocol lasting 21 weeks (P1). Then, using an updated protocol (P2), we produced an episode in 6 weeks. To evaluate the success of our previous protocols, we leveraged the action-research paradigm by polling a random sample of students across our institution to assess our podcasts on their "accessibility," "quality," and "nuance." Participants generally found the results produced by P1 more "nuanced" and "accessible." However, they reported that the results produced by P2 were of higher "quality." In response, we introduce protocol 3 (P3). We replace the previous 6-week sprint schedule with a restructured 8-week schedule, allocating more time for the research and storyboarding phases. With this schedule, we hope to increase the quality of our final recording without compromising on nuance and accessibility. Additionally, responding to feedback from our previous iteration, we are shortening the podcast to increase engagement. In this work, we discuss the efficacy of P3, and conduct another round of research to evaluate its possible impact.

Our research is supported by the Nueva School.

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Abstract 1196

Using research ethics case studies to teach about carbohydrates

Christopher Taylor, *Taft College*

Carbohydrate nomenclature and stereochemistry are standard parts of introductory biochemistry courses. However, it can be challenging to present this material in a way that students find to be relevant and engaging. These challenges are particularly relevant for institutions like Taft College that serve mostly non-traditional and underrepresented student populations. Giving students examples of problems that include both scientific and ethical components can be an effective way to create engagement. We designed a series of activities based around the Vipeholm experiment which took place in Sweden in the 1940s. In this experiment, institutionalized children were used as test subjects for the effects of sugar on dental health. The student response to these activities has been positive. Students are introduced to the Vipeholm experiments and the Belmont report during the chapter on carbohydrates. This coursework comes together in an assignment that integrates course material on carbohydrates and a discussion of experimental design and research ethics in the context of working with human subjects. By completing these assignments, students practice working with carbohydrate naming and classification, fisher projections and reducing vs. non-reducing designations. They also gain experience reading policy documents, designing experiments, and evaluating research using the guidelines in the Belmont report. These activities have been run and refined over several semesters. The discussion topics, assignments, and the changes that have been made in response to student comments and faculty observations will be discussed, as will ways to adapt this exercise to other courses and institutions.

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Abstract 1209

Development of a Bioinformatics Tool for Exploring Protein-Metal Interactions via Circular Dichroism Spectroscopy

Shanen Sherrer, *St. Mary's College of Maryland*

In the 21st century, it has become increasing important for biochemists and molecular biologists to develop computational skills that complement their experimental work. This is the result of new technologies and scientific methods that have caused exponential growth and complexity in the collection of experimental data, especially in the fields of genomics and proteomics. Fortunately, numerous computational approaches have been developed to help process and make sense of this experimental data including bioinformatics methods. Here, I describe the development of a bioinformatics database and analysis tool for studying protein-metal interactions via circular dichroism (CD) spectroscopy. Through a collaboration with computer science students to develop this online tool and testing its functionality in introductory biochemistry laboratory courses, a novel platform was created to analyze CD spectra for protein structure information. This bioinformatics tool provides interactive graphical visualizations of datasets, and it can be used for instructional purposes to help students learn about biomolecular structure and function.

This work was financially supported by St. Mary's College of Maryland, New England Biolabs, and NSF MRI grant #1919581.

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Abstract 1227

Journal Club: A Sustainable Model for Accessible Science Education and Transferability

Lauren Stoffel, *The Nueva School*

Peter Choi, Juliet Sostena, Sasha Cocquyt, Michael Sarboraria, Paul Hauser, Luke De

Journal Clubs are powerful tools to foster and encourage learning around scientific discovery. However, the limited group size, high assumed background knowledge, and professional environment often make learning inaccessible and daunting; there is a need for implementable, accessible, and sustainable Journal Clubs models. Here we present a new training and presenting model that creates a self-sustaining practice of scientific discourse. The model creates high-quality presentations and presenter trainers. With a single faculty member and three student leads, our model has trained 24 presenters a year and 10 presenter trainers. At an institution of 550 people, 50–75 non-lab members voluntarily attend our talks. Furthermore, our model is highly scalable and reproducible. Our model achieves sustainability in three ways: protocolization, focusing on outward-facing elements, and effective metrics to evaluate our success. We have developed procedures for all processes, including onboarding new members, training presenters, and dissecting scientific literature. This makes sustaining the team easy, as the protocols allow for self-propagation and also provides the opportunity to implement our system in other institutions. In our adapted model, we shifted from an inward-facing group discussion to an outward-facing group that produces weekly presentations to the broader community. This leads to a community that understands and supports our mission. Finally, we evaluate our team with a multitude of metrics. This allows us to establish feedback loops that are vital to a Journal Club's sustainability. Past literature suggests that although powerful training tools, Journal Clubs are difficult to start and their effectiveness is challenging to assess. We solve both of these issues in our model. Successful implementation of our Journal Club at the high school level indicates the potential for benefit at all levels of scientific literacy. Further, our protocols have the potential to facilitate straightforward transfer to another institution. We will assess effectiveness through audience engagement feedback, as well as through the feedback we already receive from the team. Ultimately, our results indicate that our model of Journal Club is engaging and beneficial for all students involved.

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Abstract 1239

From Poison to Medicine: Development of a Molecular Case Study

Shravya Yarlagadda, *The University of Texas at Austin*

Kristen Procko

The Foundations of Biochemistry course at the University of Texas at Austin targets upper-division biochemistry majors and focuses on macromolecular structure, enzymes, and pathways. Some of the most challenging topics for students to visualize and comprehend are protein-ligand interactions, reaction mechanisms, and kinetics. We used the common thread between these topics to author a case study for the Molecular CaseNet network. We started with the neurotoxin, sarin, and introduced medicinal biochemistry with the vivid symptoms experienced by an individual that had survived a sarin gas attack. Then, students visualized the acetylcholinesterase active site using either the PyMOL or iCn3D biomolecular modeling programs. Next, we focused on the acetylcholinesterase mechanism and concluded with an in-depth analysis of inhibition and enzyme kinetics. We based the final activity on acetylcholinesterase's connection to Alzheimer's treatment, concluding with an optimistic outlook. The development of the Poison to Medicine case study was a faculty-led project supported by Undergraduate Course Assistants; we will present case study authorship from an undergraduate's perspective. Preliminary data from student surveys indicate positive responses to the case study format, particularly regarding the kinetics portion (IRB: University of Texas at Austin #STUDY00001087).

This work is funded by the National Science Foundation Research Coordination Networks in Undergraduate Biology Education (RCN-UBE #1920270).

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Abstract 1254

Creating and Maintaining Access for Deaf and Hard-of-Hearing Scientists

Paul Craig, *Rochester Institute of Technology*

Bonnie Jacob

RIT is the home of the National Technical Institute for the Deaf, the first and largest technical college for deaf and hard-of-hearing students (D/HH). It serves more than 1100 students; about half of these students are enrolled in B.S. and M.S. programs in other colleges on our campus. RIT hosted an NIGMS RISE program from 2017–2022, with the specific goal of supporting more D/HH students to enter Ph.D. programs in biomedical, biobehavioral and clinical sciences. We are now transitioning from that research grant (which was under the NIGMS R25 mechanism) to an NIGMS training grant (T34 mechanism), with the same specific goal. As we transition, we face the challenge of providing continuing support and resources that will equip our students to succeed in their undergraduate careers, so that they can compete effectively for positions in highly regarded Ph.D. programs. Under the R25 program, we developed a series of tools to support our students: the Scientists-in-Training Series, Doctoral Readiness Meetings, Mentor Training, Lab Accessibility Evaluations, Responsible Conduct of Research Workshop, and Rigor & Reproducibility in Research Workshop. This presentation will describe how we are transitioning these tools into the T34 training program format in the midst of the pandemic and the adjustments that students and faculty have needed to make.

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Abstract 1282

Re-envisioning an equitable Biochemistry curriculum to include dialogues on anti-racism, equity, and social justice

Rou-Jia Sung, *Carleton College*

Jane Liu, Stanley Lo, Sara Hollar

As our communities (institutional, disciplinary, local, national, personal, professional, etc) continue to consider how to deconstruct systems of inequality and incorporate practices to promote diversity in an inclusive and equitable environment, it is of paramount importance that we consider how these conversations can exist within our classroom spaces as well. Here, we present a strategy for discussing the impact of racism on diversity, equity, and access with STEM and promoting social justice and ideas for action in an undergraduate biochemistry curriculum. Beginning in Fall 2020, Racism as a Public Health Emergency has now been implemented in biochemistry curriculum at multiple institutions. Here, we focus on the experiences of both the instructor and students from three successive in-person implementations at a single primarily undergraduate institution in Fall 2021, Winter 2022, and Fall 2022. These implementations included the overlay as well as a final project component in which students created their own discussion guides for future discussions in Biochemistry. The majority of students agreed that inclusion of the anti-racism overlay made the course material more relevant to them; analysis on impacts on student sense of belonging as well as instructor strategies for the development and incorporation of new activities will be presented. These activities address a critical need to reform our notion of what belongs and what does not belong in a biochemistry curriculum to incorporate issues of social justice and could be adapted to a variety of other fields.

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Abstract 1301

Honor, Duty, and Service; How Localized STEM Pipelines Can Serve our Armed Forces Veterans

Chelsea Rand-Fleming, *Auburn University*

As part of the ASBMB Advocacy Training Program, the objective of this study was to compose an institution that would serve the sole purpose of decreasing veteran unemployment rates at a local level. Veterans face an uncertain future upon the completion of service in the armed forces. Possession of hard skills and military experience does not always directly correlate to gaining employment. The result of this advocacy project was the plan to formulate localized STEM pipelines for military veterans (VPS). The institution will have several key components; local STEM company partners, STEM job boards/bulletins, pipeline degree and certificate programs, professional development sessions, and STEM pipeline veteran cohorts. This combination will result in loyal community partners dedicated to hiring veterans, assisting vets on how to market military skills and job experience, presenting education options specific to entering a career in STEM, and opportunities to find fellowship with other veterans in the local area. In culmination, the goal of VPS and the reason for its creation in all aspects is to guide veterans to long lasting careers in STEM in repayment of their service to our country, one town, one city, one state at a time. This VPS pipeline prototype once finalized and completely functional in Auburn/Opelika Alabama area, will serve as a model for other communities dedicated to providing our veterans the best resources necessary to guarantee successful lives and long-lasting STEM careers.

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Abstract 1361

Engaging the Nashville African American community in a discussion about Alzheimer's disease

Tameka Clemons, *Meharry Medical College*

In an effort to bring awareness to the seriousness of Alzheimer's disease it is important to engage the community about the disease. The objective was to hold a virtual session with the Nashville African American community in order to bring awareness about Alzheimer's disease and answer questions that the community may have. The session was held as a "lunch and learn" during the noon hour with the goal that more community members would be available to attend. Advertisement for the session was broadcasted in plenty of time in an effort to get more participants to be aware of the session. A PowerPoint slide that included data from the Alzheimer's Association and other valid sources were a major part of the PowerPoint. In addition, a personal story about Alzheimer's disease from Dr. Clemons was shared with the community. The session was well-attended and powerful as noted from the vast array of questions that were asked by the audience and the willingness of the community members to share their personal stories. The results will include the number of attendees as well as questions and clips of stories shared by the community members.

N/A.

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Abstract 1371

Modeling order metabolic pathways using beading

Debra Martin, *Saint Mary's University of Minnesota*

Metabolic pathways are an overarching and foundational concept for Undergraduate Biology and Biochemistry majors. Understanding the importance of the orderly process of these pathways and how they could be regulated can be better understood with the use of a visual modeling. Utilizing a beading technique that many students did in their youth (beading animals) as a metabolic pathway model, students investigated the necessity of order process vs random. Each student was given the role of the "enzyme" in the pathway and one group the pathway had to be in a certain order (enzyme A then B, etc) while the other group the pathway was random. Visualization of final bead project demonstrated how important order is. This technique can be used to demonstrate specificity of enzymes by having the "enzymes" randomly thread the beads or be giving a specific bead order. It can also be used for demonstrating rate limiting steps by having excess beads or limited beads for the "enzymes" to work with. Assessment of course sections that performed the activity vs sections that did not demonstrated an increase trend in comprehension of enzyme function and metabolic pathways.

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ASBMB

Abstract 1409

Quantification of Learning Advances in a Science CURE: Providing Learning Objectives to Corroborate and Validate the Advantages of Experiential Education

Arthur Sikora, *Nova southeastern University*

Course-based Undergraduate Research Experiences (CURE) seek to supply college students with hands-on experiences to develop research skills and complex thinking. As students progress through their education and enter their professional careers, improvement of these critical skills is imperative. CUREs make it possible for students to experience the enchantment of science in its authentic form by engaging in research. Regardless of race, socioeconomic status or family obligations, CUREs have been gaining popularity as an essential model to expose every student to the process of science firsthand. This study aims to gauge student learning objective mastery quantitatively using Anticipated Learning Outcomes (ALOs) designed specifically for the Biochemistry Authentic Student Inquiry Laboratory (BASIL) CURE curriculum. Likert scale-based analysis is employed to evaluate the level of content mastery student responses demonstrate. Assignment questions were designed to correspond to the most critical learning objectives. Learning gains were evaluated across several semesters and represent fully in person, hybrid and online teaching modalities. Analysis shows greater mastery of bio-informatic ALOs during remote learning. The data show that quality education can be enhanced and improved by technology. The student's mastery of wet-lab ALOs coincided with our findings that lab courses need enhanced strategies to teach critical STEM lab-research skills in an online setting. This work will allow for wider CURE adoption through quantitative assessment and instruction strategies targeted to identifying learning mastery gaps. Ultimately, research based curricula can serve as a platform to expose every undergraduate student to vital STEM research experiences.

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Abstract 1451

Comparing effectiveness of two antibodies (Aducanumab and Gantenerumab) on reducing amyloid-beta plaques

Nikhila Paleati, *Nova Southeastern University*

Akhil Godbole, Pranav Neravetla, Emily Schmitt Lavin, Arthur Sikora

Alzheimer's disease (AD) is a degenerative neurological disorder that destroys memory and other important cognitive functions. As time progresses, brain cell connections, as well as the brain cells themselves, atrophy and die. AD is caused by a missense mutation in the amyloid-beta peptide within the amyloid precursor protein (APP). The mutation results in glutamine being replaced with glutamic acid. Previously conducted studies showed that mutated forms of the amyloid-beta peptide fragment have a greater tendency to stick together and form protein clumps or aggregates. The abnormal build-up of aggregates in and around the brain cells has been found to be strongly associated with the development of Alzheimer's disease, therefore, it appeared crucial to study the methods that reduce these build-ups. Attempts to treat this disease have produced antibodies that bind to the mutated amyloid-beta peptide and clear the aggregated amyloid precursor protein out of the brain. The overall goal of this project is to use 3D printed protein models to show interactions leading to a clearer explanation of the efficacy variations between antibodies. One antibody, Aducanumab, is currently in Phase 3 clinical trials and has been fast-tracked by the U.S. Food and Drug Administration. Aducanumab functions by specifically binding to the mutated amyloid-beta peptide and clearing aggregates out of the brain. This antibody binds to a smaller linear epitope formed by amino acids 3–7 of the amyloid-beta peptide. Using Jmol, protein visualization software, the Aducanumab (6CO3) PDB was manipulated to highlight multiple hydrophobic interactions, shown in a dark salmon color, and 2 hydrogen bonds, shown in white. The small binding location, flexibility provided by fewer strong interactions, and high affinity for aggregates at a high density make the antibody ideal for clearing out large aggregates. Another antibody, Gantenerumab, is still undergoing testing in order to ensure safety and efficacy. This antibody functions by binding to a longer linear epitope formed by amino acids 3–11 of the amyloid-beta peptide. Unlike Aducanumab, Gantenerumab interacts with peptides through 2 salt bridges in addition to 3 hydrogen bonds and multiple hydrophobic interactions. Along with hydrogen bonds in white and hydrophobic interactions in dark salmon, the Gantenerumab (5CSZ) PDB was manipulated to show negative side chains of the salt bridge, labeled in red, while the positive side chains were labeled in blue. The increased number and strength of interactions reduces the flexibility of this antibody, thus making it difficult to easily bind and clear aggregated peptides. While both antibodies bind to a similar region of the amyloid-beta peptide and function to

remove aggregates, they vary in the amount and type of interactions made with the amyloid-beta peptide.

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Abstract 1457

The Impact of STEM Bridge on Belonging and Academic Confidence in Underrepresented Groups

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La Sierra University's STEM Bridge introduced academic and social opportunities to a cohort of fifteen incoming first-year students and three transfer students from underrepresented groups. Incoming first-year and transfer STEM students, especially students of color and women, experience various social and emotional challenges as they enter STEM disciplines in college. STEM Bridge aims to increase incoming students' sense of belonging as they begin college careers. Coordinators devised a meaningful two-week schedule to foster a sense of belonging for underrepresented groups. Additional objectives included giving students VIP access to campus, setting foundations for peer and faculty networking, career and internship development, and project-based learning in plant experimentation. The ultimate goal of these efforts was to help students develop a firm footing and academic confidence in STEM. Coordinators involved twenty-eight University departments in providing a rigorous two-week schedule of academic activities around sustainability. Mental health awareness activities offered a basis of emotional stability and Social engagement opportunities and allowed students to develop social bonds. Enrolled STEM students were recruited to join at no-cost two weeks prior to the fall quarter. Students were given IRB-approved pre-and post-assessments to measure impacts on growth in programming's academic, social, and emotional areas. Participants were 39% male, 61% female, and identified with the following ethnicities; 15% Hispanic, 62% Asian, 23% African American, and 23% Caucasian. Results indicated that 25% of assessed students started STEM Bridge uncertain about implementing foundational concepts in upcoming sciences courses, and 39% began STEM Bridge wondering whether their previous math classes had prepared them for upcoming courses at La Sierra University. After STEM Bridge, 93% of assessed participants felt more aware of academic and social resources on campus to support them. Assessments also indicated that STEM Bridge students gained more confidence in their ability to do research and find solutions to real-world problems. 85% of assessed students felt more confident about starting college because of the friends they made at STEM Bridge. 92% of assessed students planned to join STEM clubs after attending STEM Bridge. Assessed students also increased their awareness of mental health resources on campus by 40%. Finally, 100 percent of assessed STEM Bridge students indicated feeling more confident in their ability to be successful at La Sierra University because of attending the program. Assessment results underscore the importance of access and inclusive representation for belonging and developing academic

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confidence in STEM for underrepresented groups. The program will continue to be refined to maximize benefits for future cohorts.

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Abstract 1481

Developing a Pipeline for Authoring, Piloting, and Evaluating Molecular Case Studies

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Engaging students in molecular explorations can empower them to make connections between structure and function. Molecular CaseNet (MCN) was created to bring together multidisciplinary educators and scholars to develop and use Molecular Case Studies at the interface of Biology and Chemistry. Each case is designed to provide opportunities to visualize and analyze three-dimensional biomolecular structures relevant to the case theme, then relate the modeled structural features and interactions to functional information derived from bioinformatics resources and the scientific literature. Case studies that are currently published in MCN as Open Education Resources were collaboratively developed by biology, chemistry, and biochemistry educators, in some instances with the involvement of one or more of their students. Topics for these case studies range from various inherited and infectious diseases to evolution of biosynthetic pathways in specific plants. To support the educators and scholars in the growing MCN community, we have developed documentation and checklists that guide authors through the preparation of their case studies, with references to various professional society learning objectives related to structure and function (e.g., from ASBMB, BioCore, NIBLSE, BioMolViz). Discussions between experienced MCN members have helped establish a pipeline for piloting molecular case studies, preparing them for submission, and evaluating them to provide feedback from biomolecular visualization experts and case theme-related disciplinary experts. We present the preliminary work toward developing this pipeline, including a map of our review process, the key elements required for a molecular case study, and strategies to adapt cases for different learning environments. We invite instructors of all levels of biology and chemistry to explore our repository of molecular case studies, utilize them to teach structure-function relationships, and contribute to the pipeline by providing feedback about the use of these case studies in diverse classrooms.

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Abstract 1496

The Exploration of Yeast Alcohol Dehydrogenase Thermal Stability in the Undergraduate Biochemistry Laboratory

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The enzyme yeast alcohol dehydrogenase (YADH) is used in the undergraduate biochemistry teaching laboratory at Miami University. YADH was chosen because it is available commercially, easily expresses in *E. coli*, and can apply knowledge from lecture to the laboratory setting. The main goal of this course is to teach students essential biochemical methods and concepts by comparing commercial enzyme to overexpressed YADH. We developed a new set of experiments for the students to explore the thermal stability of this enzyme. Investigation of the stability of this protein during these experiments is designed to link to misfolded and aggregated proteins, which are associated with diseases such as Alzheimer's disease, Creutzfeldt-Jakob disease, and amyotrophic lateral sclerosis. These diseases are of high interest to researchers, so learning the methods to study protein aggregation is a valuable tool in undergraduate biochemistry. The experiments consist of (1) secondary structure analysis of circular dichroism data of YADH incubated at 10 and 55 C and (2) UV-VIS spectroscopy measurements and analysis of YADH incubated at different temperatures, 10, 25, 37, and 55 C. The objective of the first experiment, circular dichroism data analysis, is to evaluate how the secondary structure of proteins is affected by changes in temperature, which ultimately leads to disfunction of the protein. The intent of the second experiment is to use absorbance at 310 nm to monitor aggregation-associated turbidity as a function of temperature and time. Here we present the experimental design, optimization, and implementation into the undergraduate biochemistry teaching laboratory at Miami University.

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Abstract 1505

From Pandemic to Providence: The Success of the S-STEM Mentored Pathways Program Post-Pandemic

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Southwestern College (SWC) and San Diego State University (SDSU) are both designated Hispanic-serving institutions serving the San Diego region. SDSU is the top transfer destination for SWC students, and SWC is one of SDSU's three major sources of transfer students. For many students, SDSU is the only transfer option due to geographical and financial limitations. However, SDSU only admits around 20% of their transfer applicants, making the process highly competitive. Mentored Pathways from Community College to Graduate School and Chemistry Careers (the Mentored Pathways Program) aims to streamline this process via a large (~$5 million) grant through NSF's Scholarships in Science, Technology, Engineering, and Mathematics (S-STEM) program. The long-term goal of this project is to determine best practices to increase student success via mentoring and other interventions. As such, the grant supports chemistry and biochemistry students in their academic careers starting at SWC and continuing through SDSU for baccalaureate and graduate degrees. Support is provided to students in the form of scholarships and mentoring both for academics and undergraduate research. The first two years of Mentored Pathways faced the unexpected challenges of a global pandemic and world-wide switches to remote modalities. Unable to offer in-person enrichment opportunities to our students, we utilized the unprecedented plethora of webinars and online conferences to enhance our program and drive student interest. As in-person restrictions lifted, mentors started to plan small gatherings for their mentees, fostering intimate communities that allowed our students to thrive. Now that most restrictions are lifted, our students are more active than we had anticipated at the start of this program, with a large percentage active in summer research programs, and several presenting at conferences across the United States. Here we present the lessons learned from the previous three years, including the interventions and activities that we found effective at boosting student success and STEM identity. We will also share the surprising benefit of having to rely on virtual events, and the pandemic-related changes we will keep moving forward.

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Abstract 1506

Advocacy to address postdocs' needs and challenges at Brigham and Women's Hospital

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As part of the ASBMB Advocacy Training Program, I advocated for improved working and living experiences for postdoctoral fellows (postdocs) at Brigham and Women's Hospital (BWH). BWH's world-class research is driven by the 750 dedicated postdocs tirelessly performing experiments, running clinical trials, applying for funding, training the next generations of scientists, and at the same time, producing high-impact scientific articles. However, BWH postdocs are also experiencing financial insecurities, insufficient professional development, lack of job security and career advancement, and – for international postdocs, lack of transparency in visa sponsorship. These stresses have led to increased burnout among postdocs, forcing many, especially parents and those from underrepresented minorities, to quit the research they love. As the co-chair of the postdoc advocacy committee at BWH, I worked with a dedicated team to carry out the first annual survey of postdocs' needs and challenges. The lessons I acquired from ASBMB Advocacy Training Program ensured that the survey was inclusive, covered critical areas of need, and reached all postdocs at BWH. After 1 month, we obtained responses from 315 postdocs (42% response rate). As predicted, postdocs expressed the greatest stress over financial insecurities. On average, the monthly spending exactly matched the after-tax income, leaving little for emergencies or savings. Unexpectedly, 28.9% of postdocs reported being paid below BWH's required minimum, which is already 10–20% less than many neighboring institutions. Postdocs also expressed concerns over the lack of structured mentoring and professional development, with 78.6% reported not having done an annual performance review in 2022. Other concerns include perceived second-class employee status, visa sponsorship delays, and a lack of community spirit, PI oversight, and work-life balance. With this data, our advocacy committee is facilitating discussions between the BWH postdoc community and BWH leadership to brainstorm and implement changes. Our data will also assist our collaborative efforts with other postdoc associations in Boston to drive regional and national changes. Most postdocs would agree that they are passionate about solving biological puzzles and finding treatments to alleviate human suffering. This work - designing, analyzing, and disseminating the survey data, convinces me that postdocs can have the same passion and impact when directed toward our own burdens.

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Abstract 1531

Facilitating the collaborative scientific process through an interdisciplinary undergraduate protein modeling course

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An instructional model has been developed at Nova Southeastern University (NSU) through the Farquhar Honors College, whereby undergraduate students gain experience in protein modeling to describe a molecular story early in their undergraduate studies. Initially run as an independent study course for a few students at a time, the experience developed into an interdisciplinary course with co-taught by faculty from the Department of Biological Sciences and the Department of Chemistry and Physics. Coursework was developed with support from protein modeling workshops (https://3dmoleculardesigns.com/) and the NSF-funded CREST (Connecting Researchers, Educators, and STudents) Program https://crestresources.org/. This course is available to students regardless of major, academic level, or previous college-level experience and has run for two semesters (Fall 21 and Fall 22). In Fall 2022, 14 students registered for the course: 14% were freshmen, 36% were juniors, and 50% were seniors. The 14 students belonged to 5 major programs: Biology (58%), Neuroscience (21%), Chemistry (7%), Marine Biology (7%), and Psychology (7%). Five project groups (of no more than 3 students each) were tasked with researching and developing a molecular story that would be enhanced by a 3D-printed model. Each group was composed of students from varying academic levels and majors to facilitate a collaborative, project-based team learning approach. Students were provided a course-based research experience using various protein-focused bioinformatics tools: Jmol, PyMOL, Autodock Vina, and the Protein Data Bank. Six undergraduate peer mentors who previously participated in a protein modeling project assisted each group with accessing protein modeling tools, and in how to use protein modeling to tell a molecular story. Of the fall 2022 peer mentors were 67% seniors and 33% were juniors. Class sessions and assignments were modeled after research lab experience at the undergraduate and graduate levels. Students presented their progress several times during the semester and received feedback on their projects from instructors, peer mentors, and classmates All finished projects included a protein model description sheet, poster, oral presentations, Jmol script, and 3-D printed protein model. Fall 2022 Student projects included molecular stories related to Alzheimer's Disease amyloid plaque antibodies as well as BACE1 inhibitors, MCAD deficiency, PCSK9 inhibitors, and ω-conotoxin interactions with voltage-dependent calcium channels. Primary literature, course materials, and protein modeling tools provided students with the ability to learn the scientific process, apply it to understand

molecular mechanisms, and present their model descriptions to others. To better understand student learning gained as part of this experience, an altered RISC (Research on the Integrated Science Curriculum) Survey was administered at the end of the semester. Students showcase their models and protein stories to other researchers through the NSU library-sponsored website: https://nsuworks.nova.edu/protein_modeling_reports/. Many past participants have presented their work at local, national, and international conferences. This course represents a successful example of a course-based undergraduate research opportunity (CURE) that can be replicated in a wide variety of institutions and provide research opportunities for many students.

This work was made possible by funding through the National Science Foundation, Division of Undergraduate Education (NSF-DUE) grant number 1725940 for the CREST Project. Nova Southeastern University's Farquhar Honors College and Dept. of Biological Sciences also provided support. Protein model printing was made possible by 3d Molecular Designs.

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Abstract 1536

Evaluating Biomolecular Visual Literacy: A Library of Classroom-Tested Assessments for Instructor Use

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BioMolViz is a community of instructors and assessment experts working to evaluate and improve visual literacy in the molecular biosciences. For a decade, the team has supported biomolecular visualization instruction by establishing a Framework (biomolviz.org/framework) and developing NSF-funded workshops that train instructors to write assessments. The Biomolecular Visualization Framework outlines overarching themes, learning goals, and learning objectives for the targeted assessment of visual literacy, and was crafted collaboratively with input from the biochemistry and molecular biology (BMB) education community. BioMolViz workshops train instructors to use the Framework for backward design of assessments that probe students' visual literacy skills. Through this work, we have developed a five-step process for assessment validation involving iterative revision and expert panel review. Here, we report on the validation of 15 assessments that have undergone the classroom testing stage of our process. Assessment items were distributed to students at several institutions to broaden the range of instructional contexts and types of courses surveyed. We present analysis of the data from our field testing, including student responses regarding perceived difficulty and open-ended feedback. These assessments are among the first available in the BioMolViz library, a repository designed to increase instructors' access to validated visual literacy assessment tools. We will demonstrate its key features and invite the BMB educator community to use and contribute to the library.

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Abstract 1576

Lessons Learned: Supporting Blind/Visually Impaired Students in Biochemistry

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Though the chemical structures in biochemistry are too small to see, teaching biochemistry relies a surprising amount on visual aids. These can feel daunting to the students and teaching team to adapt to be accessible to a blind or visually impaired person. Perhaps, even more daunting is adapting experiential learning opportunities like labs. However, these learning materials and experiences are essential to deep learning of biochemistry. We present lessons we've learned working with a blind biochemistry student through his journey as a Biochemistry and Biophysics major. We take a student-centered approach to creating accessible content and cover tips and considerations for adapting lecture visual aids, lab data, third party software and institutional support. We seek to bring inclusion for blind and visually impaired students in our classrooms by reducing the activation energy needed to include these students fully in biochemistry learning.

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Abstract 1589

ICABL Workshops as a Model for Diversifying Leadership and Representation in a Community of BMB Educators

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Professional learning communities unite practitioners who share common goals and interests. In turn, these learning communities provide resources and opportunities to their members. The demographics of these communities, however, often can be largely homogeneous in terms of the backgrounds of the members and the institutions they represent. One of the explicit goals of ICABL is to foster the development of an Inclusive Community for the Assessment of BMB (Biochemistry and Molecular Biology) Learning by engaging individuals from historically marginalized institutions [e.g., Historically Black Colleges and Universities (HBCUs), Hispanic Serving Institutions (HSIs), and Tribal Colleges and Universities]. Funded by the National Science Foundation, ICABL hosts virtual and in-person workshops around the United States that discuss and demonstrate effective practices in assessment to BMB educators. Here, we describe the origins and evolution of our model for fostering intentional partnerships with workshop hosts and a train-the-trainer program that can diversify the membership and leadership of an organization. This model offers a roadmap for both broadening representation and building capacity for leadership within a professional learning community.

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Abstract 1591

Circular medicine. a paradigm of sustainability and resilience in education and professional development

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Circular economy is a model of economic, social and environmental production and consumption, aimed to build a sustainable society in tune with the available resources. In the Brundtland Report, (1987, United Nations) sustainability it is defined as an approach focused on "meeting the needs of the present without compromising the ability of future generations to meet their own needs." The European Medical Association (EMA), that has currently more than 50 000 members, is involved in the advancement of health, pursuing educational and professional goals addressed to the quality of environment, lifestyle and civil society through a circular medicine approach. This is a comprehensive strategy, integrating best medical practice, bioinformatics and molecular biology with economy, artificial intelligence and machine learning. Circular Medicine is the resilient framework linking all these topics with circular economy methods. Innovative possibilities of circular medicine are many: it is a comprehensive paradigm shift, countering a non-medicine based on simplistic algorithms with guidelines of insufficient effectiveness, reliability and generalizability, often passed off as innovative Artificial-Intelligence approaches. We launched in 2020 a still ongoing survey aimed at the dissemination of information and at a greater sensitivity of all our members and followers on these topics, striving to build circular medicine in the real-world. This position document is based on its preliminary results: 1. Studying and investigating the links among health, climate change, biodiversity, circular economy, robust Artificial Intelligence and Machine learning support are goals and needed practice suitable to be pursued by EMA and other medical and scientific associations. Circular economy will be fruitfully used in health facilities, including hospitals, with health professionals and life science researchers acting also in the role of influencers and opinion leaders. 2. The interventions based on epidemiology, environmental sciences, best practice in medicine, sustainable technologies and molecular biology, the pillars of a potential framework of circular medicine, need a robust bioinformatics and Artificial Intelligence support. 3. Academic curricula and health professionals CME courses should provide stronger digital knowledge, reliable procedures trainings and expertise along these lines. Daily routine of clinical observation and participatory dialogue are key elements for the progress toward a culture, practice and accomplishments of circular economy and medicine. 4. Molecular biology, still the most innovative field of medical and life science, has a role for strengthening the pathways of the circular economy, as a reliable and resilient basis of "circular medicine." 5. The lessons of COVID19, the ongoing battles for healthier lifestyles dealing with nutrition, exercise and against alcohol and cigarette smoking, and the concept of smart cities are some of the cornerstones of the proposed strategy for a real-world circular medicine. Debunking interventions against the misuse of scientific and medical are means for the development of circular economy and medicine. Effective roadmaps, guidelines and grids for recognizing and counteracting the overlap of bullying, imposter's fear, insufficient expertise and knowledge, fake assertions and evaluations are actively developed by EMA's ad-hoc workshops. AI knowledge and skills should be implemented within innovative molecular biology and medical best-practice academic-CME curricula.

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Abstract 1597

An analysis of Course-based Undergraduate Research Experience efficacy at community colleges

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Course-based Undergraduate Research Experiences (CUREs) have emerged as a well-supported high-impact practice for improving both retention of students in science, technology, engineering, and mathematics (STEM) disciplines and overall achievement of students in STEM coursework. While considerable scholarship has been performed on the overall efficacy of CUREs as a teaching approach, comparatively little work has been done to uncover the more nuanced data on how CUREs serve students at two-year institutions, especially students in minoritized groups (MGs). In this study, we leveraged the Malate Dehydrogenase CURE Community, a CURE network focused on bringing research experiences in protein biochemistry to undergraduates, to collect data on student achievement at two-year institutions. Using a validated assessment instrument, we considered how the MCC CURE experience effected the overall scientific literacy of students enrolled in the CURE at two-year institutions when compared to students enrolled in control classes with traditional labs. Our data revealed that the CURE approach to instruction was especially effective with respect to MG student scientific literacy relative to control students. Importantly, we noted that two-year college students exposed to a CURE experience were 24% more likely to be retained in the STEM disciplines relative to their peers. Taken together, the data suggest that CUREs may present an effective means for the providing of equitable opportunity for scientific retention and success for students at two-year colleges. The outcomes of this research will hopefully enable these institutions to provide biochemistry-focused high-impact educational experiences for their undergraduates tailored to enable them to join a well-equipped next generation of life scientists.

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Abstract 1617

Leveraging, Enhancing and Developing Biology (LED-BIO) Scientific Societies Shedding Light on Persistent Cultural Challenges: A Research Coordination Network

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Each field in the sciences has historically been represented by its own scientific society, bringing together individual researchers for regular meetings where they can network, share discoveries, and collaborate. While it is rare for multiple distinct scientific societies to interact, they often perform similar functions, such as promoting the professional development of their members and promoting diversity among the next generation of researchers in their respective fields. We have established a collaborative network of experts to identify evidence-based inclusion strategies to accomplish the following: (1) collect consistent demographic data of society memberships, (2) better integrate scientists in transitional career stages into scientific society activities, and (3) diversify the ranks of scientific society leaders. By fulfilling these goals, LED-BIO aims to overcome persistent challenges that frequently undermine diversity efforts within independent communities of scientists and to broadly share this information for the benefit of all scientific communities. This project is led by the Alliance to Catalyze Change for STEM Success (ACCESS), the Quality Education for Minorities Network (QEM), the Marine Biological Laboratories in Woods Hole, and the NSF INCLUDES Aspire Alliance. The LED-BIO network gathers data and crowdsources information through a series of “town hall” and “think tank”events, a methodology regularly used by QEM to bring together stakeholders to find solutions to challenges faced by Minority Serving Institutions (MSIs). During the first town hall and think tank series of the project in 2022, LED-BIO arrived at a set of barriers, resources, and interventions relevant to each of the three project goals. We share the progress made during the first year and a half of the project as well as next steps.

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Abstract 1624

Global Trends in the Determination of Coronavirus PDB Protein Structures

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Mary Boldyrev, David Roman, Matthew Mcdevitt, Janet Gonzalez, Cristina Clement

This study aims to examine the international publication patterns of coronavirus protein database (PDB) structures, beginning when the first coronavirus virion PDB structures were published in 2002 to the present (2022). Sources of these depositions were extracted from their publications and used as indicators of how countries reacted to the pandemic through research output and were then compared to demographic and economic metrics. Of the approximately 40 countries examined, the United States, United Kingdom, and China had the highest number of proteins, demonstrating research productivity centeredness in highly developed countries. These countries all displayed a peak in protein depositions in 2020 or 2021, and slowed down in 2022 likely due to the peaking of the pandemic and a slowing necessity of response. Population size was found to contribute little to no factor in determining the number of coronavirus protein depositions while higher economic status measured by the GDP per capita did correlate with higher numbers of protein depositions (Jaffe et al, 2020). The number of confirmed Covid-19 cases showed a positive association with the number of PDB depositions per country, specifically in the United States. Furthermore, South Africa and Brazil, despite not being in the top 10 research-producing countries, had a high number of cases and PDB depositions, suggesting the strong impact of confirmed cases on coronavirus research output (Normile, 2022). This study's measure of how countries' economic status, population, and confirmed coronavirus cases affects their responses in terms of coronavirus protein research output suggests an unequal distribution in publication origins, which poses a challenge to global pandemic response coordination. This study continues an earlier study presented at the PDB50 – ASBMB on-line meeting, on May 4–5, 2021 by Janet Gonzalez, Matthew K. McDevitt, David Roman, & Manfred Philipp.

NA.

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Abstract 1627

ASBMB at KU: Establishing our student chapter and enriching our community in a post pandemic world

Christopher Kywe, *University of Kansas*

Albert Park, Alexandra Coveney, Kai Smith

As a newly founded organization, establishing our presence on campus as well as finding opportunities to display the joys of biochemistry outside of the university remained challenging. In spite of the difficulties, with a year's worth of experience, our organization has managed to make an impact among our members and the Douglas County Area. Whether it's bringing in guest researchers to describe their projects or going to local schools to perform demonstrations of biological phenomenon, the University of Kansas Student Chapter of the American Society for Biochemistry and Molecular Biology (KU ASBMB) works to foster enthusiasm in both the biological sciences and the importance of community engagement. Our organization began in the Spring 2022 semester. While we held meetings during this time, the restrictions on organizational meetings coupled with the decline in extracurricular engagement resulted in low attendance. However, with the return to in person classes during the Fall 2022 semester, our organization prioritized recruitment to bolster our numbers. By attending biology department introductory events as well as tabling our organization, we were able to increase attendance during meetings. After recruiting our members, we planned monthly meetings. During these meetings, we conducted professional development workshops to help our members attain both the knowledge and skills required to succeed as they progress through their academic career. From hosting workshops to get students involved in undergraduate research to identifying opportunities to pursue during the summer, members could walk away from each meeting with knowledge of opportunities to help them get closer to their goals. Finally, outside of the monthly meetings our organization emphasized community outreach. Initially, we faced some challenges in contacting elementary schools. After continued persistence, our organization was able to host science nights in conjunction with many other organizations such as KU's Chemistry Club. During the fall semester, the chapter tabled at the Natural History Museum during the annual Macabre at the Museum, in which we extracted DNA from various fruits and dissected owl pellets. Through our outreach, we aim to get children interested in biology through experiments outside of the classroom setting. KU ASBMB aims to continue these efforts indefinitely. With the recruitment of new members, we have ensured that our legacy will continue even as the founding members graduate. Beyond the community outreach and professional development opportunities that our organization provides, we hope to be a model organization that inspires others to found student chapters.

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Abstract 1638

Integrating Public Policy Education in the Biochemistry Curriculum

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Yasmeen Williams

Increasing emphasis on science-for-policy and policy-for-science creates numerous opportunities for scientists and policymakers to collaborate on issues of national importance. Formulating a sound policy requires a thorough understanding of the policy process and analysis supported by scientific evidence. Public Policy education is rarely included in higher education science curricula, and science graduates need to be more educated about public policy. Here we make a case for conceptualizing policy education as an interdisciplinary subject integrated into undergraduate science curricula. Translating disciplinary knowledge into cross-curricular pedagogical content has different layers of complexity, ranging from resistance to changes in curriculum and allocation of disciplinary specialists. Here, we provide a framework for designing and implementing policy education in an undergraduate biochemistry curriculum. We provide sample curriculum content, learning objectives, and outcomes to introduce and engage science majors in the policy process.

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Abstract 1756

A Survey of Accredited Programs: Feedback on ASBMB Accreditation and Certification Exam

Jennifer Loertscher, *Seattle University*

Kirsten Block, Daniel Dries, Peter Kennelly, Victoria Del Gaizo Moore, John Tansey, Ludmila Tyler, Adele Wolfson

ASBMB accreditation provides an independent, outcomes-based valuation mechanism that recognizes excellence in B.S. or B.A. degree programs in biochemistry and molecular biology and related disciplines. Since 2013, 105 programs from 38 states have been accredited, representing a diverse range of public and private institutions. Accredited institutions have access to an independently constructed and scored instrument, the ASBMB certification exam, for assessing student achievement and program effectiveness. To explore the benefits and barriers of both accreditation and the certification exam from the perspective of the programs, a survey was sent to all currently accredited programs (100 programs) with 50 programs returning fully completed surveys. Responses indicated that programs find information and support provided by ASBMB during the accreditation process to be clear and helpful. Accreditation is most frequently used by programs for self-review, for review by their units or institutions, and for external review. Overall, programs reported that the process was worthwhile, and that it led to collection of data that would not otherwise be examined. Of the programs that responded, 86% offered the certification exam to students and identified a variety of benefits including satisfying requirements for independent assessment and providing students an opportunity to document their proficiency in the discipline and to demonstrate their performance relative to peers at other institutions. Barriers identified include cost and timing of the exam. Survey results in tandem with feedback from the broader BMB community continue to inform how the accreditation process and certification exam are developed and delivered moving forward.

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Abstract 1765

A laboratory sequence linking Organic Chemistry II and Biochemistry Laboratories

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Our department's curriculum includes courses in general, organic, analytical, inorganic, physical, marine, and biochemistry. Often, students take one course after another without seeing how they blend to form the bigger picture. Students frequently say they wish they realized how the courses related to each other earlier in their academic career. Our goal in this project is to design a lab project that bridges the organic chemistry and biochemistry subdisciplines. Currently, Biochemistry Laboratory consists of a 10-week long project that creates a research-like feel that students enjoy based on data from student perception surveys. In this project, we intend to enhance that experience by using student-generated compounds synthesized in Organic Chemistry II Laboratory; students will prepare analogs of L-phenylalanine in that course. Students in Biochemistry Laboratory will analyze the effectiveness of those compounds in inhibiting the enzyme calf intestinal alkaline phosphatase. Many students take these two courses in consecutive semesters, and we hope that several students will be able to synthesize a sample one semester and test it during the following semester. This experience will provide an example to students of how chemistry projects are linked in the real world. For instance, pharmaceutical and organic chemists often work together with biochemists in designing drugs, which are then tested for the effectiveness in targeting a protein. This project may provide students with an opportunity to see connections they might not otherwise realize exist. In this poster, we will present student-generated data from pilot experiments, as well as pedagogical information.

This research was supported by a University of Tampa Research Innovation and Scholarly Excellence Award/David Delo Research Grant (CEB, DSW).

103514, https://doi.org/10.1016/j.jbc.2023.103514

Abstract 1773

Reexamining the NSF GRFP review process

Roxanne Evande, *University of Delaware*

As part of the ASBMB Advocacy Training Program, I aimed to find ways the National Science Foundation Graduate Research Fellowship Programs (NSF GRFP) application could improve to ensure equity in their fellowship awards and stay in line with its main goals. In the United States, securing federal research funding is a top priority for most graduate students and understandably so, graduate fellowships provide financial security, prestige, and a valuable network. The National Science Foundation (NSF) offers one of the most competitive graduate research fellowship programs (GRFP) that has awarded several Nobel laureates and leaders in the science and engineering world. However, in late July 2022, policymakers on Capitol Hill sent a letter to the NSF director, expressing concerns about the importance of your agency's mission and the type of grants that were being funded. The NSF GRFP aims to award individuals that demonstrate potential to be high achieving scientists and engineers, and to broaden participation of underrepresented groups. As policymakers scrutinize the NSF's budget and grant awarding process, the NSF must begin looking at its various grant and fellowship review processes to ensure they are sticking to their overall missions' and working to thoroughly eliminate biases. Two surveys were conducted using QualtricsXM and distributed via Instagram, Twitter, LinkedIn and organization emails. The first survey was completed by applicants that received the fellowship, an honorable mention or did not receive the fellowship. The second survey was completed by reviewers that had reviewed NSF GRFP applications at least once. The responses were analyzed and used to inform three policy recommendations to improve the peer review process for GRFP, ensuring fairness and equity. A person's potential to become a high achieving career professional should not be based areas that individuals with better resources will excel in.

103515, https://doi.org/10.1016/j.jbc.2023.103515

Abstract 1794

STEM identity at a rural primarily undergraduate institution

Alexa Neiderer, *Juniata College*

Susan Gembic, Alexis Hammaker, Daniel Dries

In their pioneering work (J. Res. Sci. Teach., 2007), Heidi Carlone and Angela Johnson describe a person's science identity as comprising three dimensions: performing like a scientist, being recognized by oneself and by others as a scientist, and having content knowledge within a science discipline. Carlone and Johnson also recognized that other social constructs, such as gender and race, can influence one's science identity. Here, we ask how students identify their peers as STEM students or non-STEM students. Using grounded theory, we identify themes from conversations with focus groups composed of both STEM and non-STEM majors. We also capitalize on the rural setting of our small liberal arts college to ask how rurality influences students' identification of their peers. Moreover, we examine how rurality influences science identity within this same context. Together, these analyses expand the literature on science identity, particularly within the context of rurality.

103516, https://doi.org/10.1016/j.jbc.2023.103516

Abstract 1878

What Does Educating Biochemists Really Mean?

Regina Stevens-Truss, *Kalamazoo College*

How does one write an abstract and give a presentation for an award that one was nominated and selected for? Well, this has been my dilemma since receiving the news that I was the recipient of the 2023 ASBMB Award for Exemplary Contributions to Education. Humbly, I have decided to tell my story. I joined the chemistry department faculty at Kalamazoo College in January 2000 as one of two biochemists. Kalamazoo College is a small (~1450 students) liberal arts college in SW Michigan, and is part of the Great Lakes Colleges Association (GLCA). In 2000 our Chemistry Department consisted of six faculty (two in biochemistry and one in each of the other sub-areas of chemistry) and we graduated ~15 chemistry majors per year. Today, our department has seven full-time tenure track faculty (two in biochemistry and two in organic chemistry), we graduate ~35–40 majors per year, and we are now the Department of Chemistry and Biochemistry. In addition to developing the new biochemistry major, I have engaged in multiple ways with moving how we approach teaching biochemistry forward, at least in ways that for me guide true learning. We all can agree that the general understanding of biochemistry is complicated by the overwhelming variety of life that exists on earth, let alone the amounts of ins-and-outs and undulations in a cell. While general biochemistry courses focus primarily on mammalian cell chemistry, the biochemists of the future need to be able to relate that knowledge as the basis for understanding other organisms. In my presentation I'll take you through my transitions to teaching a 10-weeks survey biochemistry course to developing a major, all with an eye towards Teaching & Learning, and with equity and inclusion as my guiding light.

103517, https://doi.org/10.1016/j.jbc.2023.103517

Abstract 1884

Building Biochemistry Content Knowledge from the Primary Literature

Natalie Slusser, *Juniata College*

Kara Mette

Due to the vast expanse of biochemistry and molecular biology (BMB) content, modern BMB curricula have shifted toward competencies and skills, as outlined in Vision & Change (AAAS, 2011) and in ASBMB's Foundational Concepts. Instructors, however, can still tend towards didacticism in helping students demonstrate these competencies and skills. What if an instructor had students construct their BMB knowledge by trying to decipher primary literature? Would students be able to demonstrate the competencies and skills required of them in a modern BMB curriculum? Here, we present CIMMPL, a pedagogy that uses the Collaborative Interrogation and Meaning-Making of Primary Literature. We map competencies and skills to a set of primary literature articles and demonstrate student proficiency. We also evaluate the student experience in the CIMMPL classroom using deductive coding against Self-Determination Theory and grounded theory. Results show that CIMMPL can not only build students' competence, but also encourage autonomy to develop independent and inquisitive learners.

103518, https://doi.org/10.1016/j.jbc.2023.103518

Abstract 1895

"It's completely erasure": A Qualitative Exploration of Experiences of Transgender, Nonbinary, Gender Nonconforming, and Questioning Students in Biology Courses

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Aramati Casper

Biology is the study of the diversity of life, which includes diversity in sex, gender, and sexual, romantic, and related orientations. However, a growing body of literature suggests that undergraduate biology courses focus on only a narrow representation of this diversity (binary sexes, heterosexual orientations, etc.). In this study researchers take the approach of listening to the experiences of students and learning from them by interviewing transgender, nonbinary, gender nonconforming and questioning students about their biology courses. The interview questions focused on the messages about sex, gender, and orientation they encountered in biology and the impact of these messages on them. We identified five overarching themes in these interviews. Students described two narratives about sex, gender, and orientation in their biology courses that made biology exclusionary. These narratives harmed students by impacting their sense of belonging, career preparation, and interest in biology content. However, students employed a range of resilience strategies to resist these harms. Finally, students described the currently unrealized potential for biology and biology courses to validate queer identities by representing the diversity in sex and orientation in biology. I will describe approaches suggested these students as well as approaches suggested by gender studies scholars for making biology more queer inclusive.

Aspects of this work were funded by the National Science Foundation (NSF AWD 2018693, 2201808).

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Abstract 1914

Reshaping active learning by using computational tools in undergraduate biochemistry education

Bonnie Hall, *Grand View University*

Active learning has been shown to improve retention of chemistry knowledge and to support inclusivity in the classroom. A way to enhance active learning in biochemistry is by incorporating the use of one or more computational tools. Many tools can be easily aligned with existing course content for brief computational activities to emphasize or explore key concepts. A range of tools and curricula are also available that are appropriate for a more in-depth integration throughout a course or as a major course project. The most intensive option is to design or re-design a course to focus heavily on using computational tools, with published curricula already available for several biochemistry-based course topics. There are even tools to allow students to perform predictive modeling using machine learning without requiring that students learn a coding language. These tools and curricula will be discussed for various levels of integration, along with examples of how they can be used for a variety of biochemistry topics and courses.

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103520, https://doi.org/10.1016/j.jbc.2023.103520

Abstract 1930

Assembling The Pieces: Analyzing the Visual Literacy Skills of Horizontal Translation Representations of Undergraduate Students Across the Chemistry and Biochemistry Curriculum

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Vanessa Andrade, Chloé Morin, Xavier Prat-Resina, Cassidy Terrell

The development of visual literacy skills in undergraduate students has the potential to increase the interpretation of external representations and aid in the development of conceptual understanding. To date, there is very little visual literacy research addressing the capabilities of students in interpreting and storing information, and how students organize this information in their neural networks. This study aims to analyze both qualitative and quantitative data on expert and student visual literacy skills with respect to horizontal translation skills. More specifically, we will determine how students' structural knowledge and organization of horizontal translation representations in enzyme-substrate and oxygen-binding concepts evolve across the chemistry and biochemistry curriculum. In order to do this, students and experts took surveys that contained different visual representations and were asked to rank them according to relatedness. The Pathfinder program was used to obtain the average neural networks and quantitative values such as degree, eccentricity, Path Length Correlation (PLC), and Neighborhood Similarity (NS) values, as well as any patterns of chunking or organization of the neural networks. Rather than looking at individual data, the datum was averaged by course via the Pathfinder program, which allows for the observation of progress, or changes, depending on the course in which the students are enrolled across the curriculum. The degree values look at the nodes that are the most branched, or those which appear to be the most connected to other concepts. The eccentricity values look at the most central node, or what is deemed to be most closely connected to other concepts, and this is indicated by a lower value. The PLC is the relatedness of two nodes between the average experts and students, with a score close to 1 indicating high correlation and a score close to 0 indicating low correlation. NS measures the way nodes are grouped, with ranges of 0 (low similarity) to 1 (high similarity). Furthermore, looking at patterns of chunking or organization in the average neural networks allow for similarities of node groupings to be observed among the experts and students. The average student neural networks and quantitative values were analyzed in reference to the average expert neural network and quantitative values. These findings may provide insight into how student organization of knowledge and visual literacy skills are acquired and maintained, which can be helpful in aiding educators to adapt their curricula to further support student learning in relation to scientific topics.

103521, https://doi.org/10.1016/j.jbc.2023.103521

Abstract 1931

Innovating Life Sciences Laboratory Training: Molecular Biology Laboratory Education Modules (MBLEMs) as a Model for Advanced Training at Diverse Institutions

Melissa Srougi, *North Carolina State University*

Anita Corbett, Christina Garcia, Michelle Sabaoun, Maria Santisteban, Vijay Sivaraman, Stefanie Chen, Carlos Goller, Robert Kelly

Keeping pace with recent scientific breakthroughs and incorporating such developments into the educational setting is challenging from both economic and instructor time perspectives. Yet, educational strategies that rely on lecture alone or traditional, but outdated, laboratory experiences ineffectively prepare the current and future generations of students that will comprise the US biomedical workforce. The higher education community would benefit from a flexible and dynamic paradigm that conveys up-to-date molecular biology laboratory training to current and future generations of students at a wide range of diverse institutions. At NC State University, the campus-wide interdisciplinary Biotechnology Program has developed cutting-edge, flexible, inquiry-based Molecular Biology Laboratory Education Modules (MBLEMs). MBLEMs are flexible educational units that provide a repertoire of inquiry-based lab exercises that can be used as complete courses or as parts of existing courses. Students in MBLEMs engage in the most recent experimental developments in molecular biology to address emerging research questions. In collaboration with UNC-Pembroke, Emory University, North Carolina Central University, Alamance Community College, and Centre College, we have used MBLEMs as resources to be re-imagined to fit the unique needs of the institution involving a diverse range of learners. MBLEMs have been adapted and improved for use in a variety of courses including first-year STEM courses, genetics, and molecular biology as well as a graduate-level technical skills training workshop. Using a mixed-methods study design over the course of two years, we assessed the effectiveness of adapted MBLEMs at collaborating institutions. Students' academic performance was assessed using exams and assignments, while perceptions were assessed using a pre/post survey. Here we will discuss the MBLEM model, emerging results in student achievement of learning outcomes, and reflections from the study.

This work is supported by the National Institutes of Health Innovative Programs to Enhance Research Training grant R25 GM130528.

103522, https://doi.org/10.1016/j.jbc.2023.103522

Abstract 1933

The Bigger Picture: Examining the Organization of Structural Knowledge for the Vertical Translation Visual Literacy Skill of Undergraduate Students

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Destiny Regalia, Xavier Prat-Resina, Cassidy Terrell

Structural knowledge refers to how students file information in their minds for later retrieval and use in various situations. The learner must assimilate new material into their retrieved, prior structural knowledge of the topic to acquire further information. A learner's structural knowledge includes complex connections between concepts within a subject. It has a hierarchical structure that influences how the data is stored in long-term memory. As of yet, little research has been published examining how students analyze and store biochemical information and representations in their long-term memory. Studying undergraduate students' visual literacy skills can improve conceptual learning while reducing cognitive load. The development of vertical translation visual literacy skills in biochemistry can alleviate misunderstandings of concepts, producing better organized structural knowledge, eventually resulting in long-term retention and greater understanding. In addition, the number of tools for assessing visual literacy is limited. A study performed with undergraduate students enrolled in a general chemistry I course measured students' structural knowledge, or schema or neural networks, of topics by asking them to rank the relatedness of words/phrases. Our study aims to develop a similar tool that measures participants' schema of biochemistry visual literacy skills by asking them to rank the relatedness of biochemical representations instead of words/phrases. Specifically, this study aims to determine how students organize the vertical translation visual literacy skill of protein structure and nucleic acids into neural networks. This study was designed to identify whether there are differences in advancing towards expert-like organization between the protein structure and nucleic acids survey. Using Pathfinder, we analyzed undergraduate students' schema with variable biochemistry knowledge at one university against an expert referent network. The quantitative values relating to path length correlation, locality, neighborhood similarities, eccentricity, and degree were collected from the surveys. These values measure the consistency and alikeness of survey responses compared to the expert reference network, indicating a student's visual literacy skill on a novice-expert scale. Grouping analyses of these data indicate high, middle, and low-performing groups, additionally sorted by demographic data to establish differences in the progress of vertical translation visual literacy skills. Qualitative data includes the observable patterns and neighborhood similarities of the neural networks. The results of this study will aid the development of curricula for undergraduate students' optimal learning and retention of information.

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ASBMB

Abstract 1971

Analysis and manipulation of fluorescent proteins as a guided-inquiry classroom laboratory project

Mary Gething, *University of Tampa*

Anna McCormick, D. Witherow, L. Carastro

For the past 10 years, students at The University of Tampa have engaged in a semester-long lab exercise in their Advanced Biochemistry course (a full semester course that meets twice a week for four hours each class) where they subclone the enhanced green fluorescent protein (egfp) gene to create a GST-tagged gene, screen colonies, and purify and analyze the fusion protein. To update this lab sequence, while still exposing students to the same fundamental biochemical techniques, we have explored using variants of egfp to create a more varied student experience in a guided-inquiry model. Students are given bacteria containing a plasmid corresponding to an egfp variant. Upon DNA purification, students sequence their plasmid to identify their variant. At this point, students are expected to, with guidance from the instructor, to design a subcloning strategy to clone the plasmid into the pET-41a vector. Following subcloning, all students in the course will be working on steps concurrently, but with slightly different constructs, which facilitates the instructor in working with the course as a consolidated group. Upon purification, standard protein analysis techniques, such as SDS-PAGE and western blot, are used. Additionally, since the protein is fluorescent, students measure the spectral properties of the purified protein and compare to literature values. This lab sequence provides a great deal of flexibility for individual students, while maintaining a structured framework to allow the instructor to work with multiple students at the same time. In this poster, we present preliminary student data from these experiments, as well as details for other instructors who might be interested in adopting these experiments.

This research was supported by a University of Tampa Research Innovation and Scholarly Excellence Award/David Delo Research Grant (LMC, DSW).

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Abstract 1983

Probing stability in adenosine deaminase using site-directed mutagenesis: the design and implementation of a CURE exploring biochemical and medical implications

Geoffrey Lippa, *Alfred University*

Undergraduate research provides many advantages to students in STEM. The most apparent advantages include learning different laboratory skills and expanding one's knowledge. However, these independent experiences may not always be possible at small institutions with fewer faculty and limited resources. Course-based Undergraduate Research Experiences (CUREs) can help bypass these restrictions while also promoting inclusivity in scientific research (Bangera 2014). However, the design and implementation of a CURE can impede faculty focused on creating these opportunities for students. The CURE described here is meant to be implemented in an upper-level undergraduate course focusing on biochemistry. Severe combined immunodeficiency (SCID) is a disease that weakens the immune system, conceding to various infections. 10–20% of SCID cases originate from a deficiency in the enzyme adenosine deaminase (ADA). While bone marrow transplants are one form of treatment, others include enzyme replacement therapy (ERT) and gene therapy (Flynn 2018, Bradford 2017, Booth 2007). This CURE explores the process of protein engineering, which is often necessary to stabilize or increase efficacy in an enzyme, like ADA, for therapeutic usage (Tobin 2014). Over 12 weeks, students utilized bioinformatics, protein visualization tools, and wet-lab techniques to identify and evaluate the stability effects of an ADA single residue mutation of the students' choice. This authentic research experience is in the early stages of evaluation. Summative assessments include a poster and lab report. These are built from assignments over 12 weeks. These assignments included a research report on SCID, a purine metabolism schematic, a thermal shift assay results figure and analysis, and many more. Course evaluations included subjective questions about the implementation and format of the CURE. Several students valued the autonomy of selecting their own ADA mutation and the variety of scientific techniques presented to them over the semester. The CURE described here is closely aligned with work conducted by both the Biochemistry Authentic Scientific Inquiry Laboratory (BASIL) consortium and the Molecular CaseNet network. While BASIL evaluates proteins with a known structure for unknown functions using computational and wet-lab techniques, this lesson focuses on a target with a known function, evaluating residues of structure linked to overall function (McDonald 2019). Molecular CaseNet is tasked with the development and use of molecular case studies at the interface of biology and chemistry, connecting students to the PDB and protein visualization software for functional analysis (Goodsell 2021). This CURE combines the wet-lab techniques associated with BASIL and the protein

structural analysis software used by Molecular CaseNet activities. Overall, this CURE provides undergraduate instructors with a framework to provide an inclusive authentic research experience.

This research is funded by Alfred University.

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Abstract 2037

It's past time! Bringing climate change into the biochemistry curriculum and beyond

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Karla Neugebauer

Human-caused climate change poses perhaps the greatest threat ever faced by humanity. It exacerbates deeply rooted problems of inequality, disease burden, and food/water security. It requires a level of collective responsibility and change, from the local to global, that is arguably unparalleled in human history. Yet how often do we mention it even casually in classes, much less as a defined part of our curriculum? We believe that climate change should become part of all biochemistry classes if we wish them to be relevant to the lives of our students and to be more than just general requirements and service to the medical school curriculum. We describe examples to link climate change and biochemistry. An obvious entry point includes proteins like carbonic anhydrase, bicarbonate transporters, and carboxylases that are involved in fixing CO2. Adaptations to increased temperatures of individual biomacromolecules and key enzyme systems such as Photosystems I/II and nitrogenase are critical in a warming planet. Genetic engineering of individual enzymes or the creation of new pathways for carbon capture, biofuel syntheses, and industrial-scale manufacture of new building materials that sequester carbon will be key in mitigation. In addition, we will discuss a separate advanced undergraduate seminar course at Yale University, Biochemistry and our Changing Climate, that explores the basic biochemistry that governs living systems' response to a changing world. The course addresses the core concepts of biochemistry in a climate context. Here are some examples. Increasing temperatures lead to coral bleaching in part through peroxide produced by the stress response in the coral's algal symbionts leading to the expulsion of the coral tissue's only food source. Growth-promoting compounds are present in wood smoke (think forest fires). The basis of most herbicides (think monoculture farming) target the enzymes of the aromatic amino acid synthesis pathway. Remarkable plants like Palmer Amaranth escape death by amplifying extrachromosomal copies of the genes encoding these enzymes. Thus, molecular explanations abound to link biochemistry and climate change! Other topics of interest include 'fake meat' – what chemicals and their receptors make food taste like meat and, relatedly, how do insect vectors of zoonotic diseases choose us for their next blood meal? We will discuss a new chapter, Biochemistry and Climate Change, in the BioLibreText OER, Fundamentals of Biochemistry, that educators and students can easily use to

study climate change in a biochemical context. To use our influence as respected scientists and experienced communicators to further our efforts in climate advocacy, we will offer concrete examples to help biochemists bring the discussion of climate change and its best solutions to the greater academic and lay communities.

103526, https://doi.org/10.1016/j.jbc.2023.103526

Abstract 2057

The CRISPR in the classroom network: a support system for instructors to bring gene editing technology to the undergraduate classroom

Jay Pieczynski, *Rollins College*

Michael Wolyniak, Donna Pattison, Tiffany Hoage, Dawn Carter, Latayna Hammonds-Odie, Sara Olson, Maria Santisteban, Nicholas Ruppel

CRISPR-Cas9 technology represents a once-in-a-generation advance that allows precise gene editing and has become a mainstream technique in research. However, most undergraduate instructors do not have the training or support to integrate CRISPR-Cas9 into their courses. To remedy this, we formed the "CRISPR in the Classroom" Network and are facilitating workshops and mentoring activities designed to provide instructors with the skills, support, and confidence needed to introduce and implement CRISPR-Cas9 technology in undergraduate classrooms (NSF RCN-UBE #2120417). Our summer workshops provide participants with a flexible, easily-adapted curriculum and start-up kits to overcome the hurdles associated with implementing a new technology. Assessment data from previous workshops show that over 80% of attendees indicated that the design and organization of the workshop facilitated their understanding of CRISPR and that two-thirds of attendees had implementation plans for CRISPR-based activities for their courses within one year of the workshop. The CRISPR in the Classroom Network represents a dynamic community of practice for integrating CRISPR-Cas9 into courses and across model systems.

Funding for this project is provided by NSF RCN-UBE#2120417: Bringing CRISPR-Cas9 Technologies to the Undergraduate Classroom: An Undergraduate Instructors' Network.

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Abstract 2065

Protein Centric Research in Undergraduate Labs: The Malate Dehydrogenase CUREs Community (MCC) Faculty Network Promotes Student Collaboration to Increase Engagement

Amy Springer, *Umass Amherst*

Ellis Bell, Lisa Gentile, Joseph Provost

Malate dehydrogenase Course-based undergraduate research experieince Undergraduate research Active learning Diversity and inclusion.

We are using course-based research experiences (CUREs) to provide opportunities for undergraduates to gain exposure to laboratory research and learn hypothesis-development in protein-centric research, specifically with projects related to Malate Dehydrogenase (MDH). Through our network of faculty from around the country, the MDH CUREs Community (MCC) provides an inclusive and sustainable community organized into 3 regional hubs, and contains members from community colleges, MSIs, PUIs, comprehensive, and research-intensive institutions. Because research experience provides so many benefits to undergraduates, these faculty are teaching MDH CUREs to greatly increase access to research for a broad range of undergraduates including populations historically underrepresented in STEM. Here we discuss the benefits of collaborative CUREs. Assessment data show that the collaborative CURE approach improved students' scientific skills as well as their awareness of the importance of STEM. We present an example of a CURE using a collaborative model in a modular format (less than a whole semester) for an introductory biochemistry laboratory at a large state university (UMass Amherst). Students in the course choose a mutant of watermelon glyoxysomal MDH, make predictions about how the mutation will affect function, and present their ideas in a web conference with an MCC expert from the University of San Diego. As they further develop their hypotheses and conduct their experiments, they communicate in person with their instructors and through electronic messages with the MCC expert, including sharing their final results. In this way, even this large course (approximately 48 students per section) can have small group interactions to talk about their ideas and results. Assessment data show that the collaborative CURE approach improved students' scientific skills as well as their awareness of the importance of STEM and we report student feedback about the impact of the CURE and how their approach was changed because of having someone outside their institution who was interested in their projects. The MCC has over 70 faculty involved through workshops, collaborations and offering MDH CUREs in their classrooms, providing research experience to undergraduates from wide-ranging backgrounds. Information about how to get involved in MCC, including our summer Fellows cohort, is provided. MCC CUREs have been shown to support student learning and ownership of research, and provide a wealth of opportunities for comparative studies, access to scientific information and community to assist with course design and sharing of reagents. The additional element of collaboration provides a model for ways to present CURE research projects in the context of the greater scientific community.

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ASBMB

Abstract 2081

The Impact of Integrating Diversity, Equity, and Inclusion-related material in Biochemistry Classes

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Caela Fedraw

Students' unique backgrounds and identities can influence how they view the field of science and their personal capabilities to succeed in a complex science course. This work aims to understand how diversity, equity, and inclusion (DEI)-related material in a biochemistry class impacts student perceptions of themselves and the field of science. Pre-and post-surveys were taken by biochemistry students enrolled in at least one semester of a biochemistry class, and student responses were analyzed using IBM SPSS. A Wilcoxon signed-rank test was performed to analyze changes in student answers over time, and follow-up interviews were conducted for qualitative analysis. Student responses changed significantly from pre- to post-survey for over half of the survey questions asked. Interestingly, when data were disaggregated by ethnicity, students of non-European descent reported more significant changes over time, compared to their peers of European descent. This research suggests that course implementation of DEI-related material can impact student views and opinions of science, but varies according to reported student identity.

This work was supported by a University of Detroit Mercy Center for Excellence in Teaching & Learning Finding Innovation & Results in Education (FIRE) Grant. Funding for the FIRE grants is supported through a Department of Education (DoE) Title III - Strengthening Institutions Program (SIP) Grant (P031A200183).

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Abstract 2084

A one-week laboratory skills bootcamp increased self-efficacy, comfort level, and medical science knowledge in undergraduate students

Phoibe Renema, *University of South Alabama*

Sarah Urankar, Nancy Rice

Undergraduate research experiences enhance student persistence, self-efficacy, and performance in STEM. Often however, students do not have the skills necessary to perform in a research environment, and as such, do not seek out these experiences. To address this need, we developed and implemented an intensive one-week biomedical laboratory skills bootcamp (BLSB) specifically for undergraduate students with little or no research background in May 2022. BLSB participants received training in basic biomedical laboratory safety, technique, equipment handling, statistical analysis, and data interpretation while also being informed of career development and research opportunities. Following the bootcamp, student outcomes were assessed by the Undergraduate Research Student Self-Assessment (URSSA) tool which evaluates learning gains acquired through a research experience and includes additional affective outcomes such as motivation and self-efficacy not necessarily captured in direct skills assessments. Demographics of our students indicates that 85% of the students in the program were female, 38% of students identified as White, 31% Black, and 46% Asian (n = 13). Fifty percent of students were freshman/rising sophomores with the remaining being distributed evenly across academic level. Self-reported gains in self-efficacy for 8 items within the category of "Thinking and Working Like a Scientist" ranged from 3.8 ± 1.09 to 4.5 ± 0.78 (on a 5-point scale), with the highest gain being achieved in "understanding the relevance of research to my coursework." Self-reported personal learning gains across 8 items ranged from 4.2 ± 0.93 to 4.7 ± 0.63, with the highest gain being "comfort in working collaboratively with others." One hundred percent of the participants indicated that they were motivated to participate in the BLSB to explore their interest in science, gain hands-on experience to prepare them for research, and have a good intellectual challenge. In addition, participants were assessed pre- and post- BLSB for knowledge acquisition in biosafety procedures, equipment handling, calculations, data interpretation, and biological principles of PCR. Students' post-test scores were significantly higher compared to their pre-test scores with the greatest improvement in questions regarding biosafety and equipment handling (mean of differences 24.38,

$p < 0.0001$, $n = 20$). Together, these data indicate that students who participated in the BLSB had significant learning gains in skills acquisition, self-efficacy, and laboratory specific knowledge which led to an increased understanding of the relevance of research to their overall academic course work and future career goals. Future longitudinal studies will determine whether learning gains were maintained through graduation.

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Abstract 2089

Roadmaps of Biochemical Knowledge: Analyzing Neural Networks of Nucleic Acids and Protein Structure Formed by Undergraduate Chemistry and Biochemistry Students

Destiny Regalia, *University of Minnesota-Rochester*

Safa Sheikhibrahim, Xavier Prat-Resina, Cassidy Terrell

Undergraduate level biochemistry is an upper division course that can be difficult to understand, as it heavily relies upon visual representations. Interpreting these can exceed the cognitive load for students. The cognitive load theory explains that our preexisting frameworks are used to process and assimilate new information. Only a specific amount of information can be transferred to long term memory at a time. By exceeding this information capacity, learning is hindered as the excess acts as extraneous cognitive load. Improving students' ability to understand visual representations and to incorporate this with content could aid in assimilating new information into pre-existing frameworks. This will lessen the cognitive load and then lead to increased conceptual understanding. Students form neural networks after observing visual representations, connecting various topics with one another. As of now, there is little research regarding how students interpret biochemistry visuals as well as store the information they decode in their memory. There are also very few tools that allow researchers to assess students' neural networks, or how they organize the information they extract from visual representations. Recently, a study was conducted in an undergraduate general chemistry course where students ranked the relatedness of words and phrases to measure students' neural networks. This study created a similar instrument that measures students' organization of nucleic acid information. Rather than using words and phrases, however, students ranked the relatedness of biochemical representations. This study aims to determine if students' neural networks become more expert-like regarding nucleic acid vertical translation visual literacy skills as they progress through a sequential chemistry and biochemistry curriculum. In addition to nucleic acids, a sister survey was deployed for analyzing protein structure representational schemas. We analyzed our data with Pathfinder, a tool that allows us to examine qualitative and quantitative aspects of students' neural networks, relative to an expert referent network. The quantitative data include eccentricity, neighborhood similarities, coherency, degree values, and path length correlations. These values allow us to quantitatively compare student organization of knowledge with expert neural networks. We also qualitatively analyzed images of the neural networks of students by identifying patterns and similarities with expert neural networks to identify chunking of knowledge in relation to the biochemical representation employed. Our findings may allow educators to better develop their courses for optimal student

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learning and retention of information. Using the data to guide development of visual literacy skills will lead to increased student comprehension as comprehension is tied to understanding visual biochemical representations. Courses can then be designed to alleviate cognitive load arising from these representations, aiming for long term retention.

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Abstract 2095

Title: Weaving Through Neural Webs: Measurement of How Students Connect the Visual Literacy Skill of Horizontally Translating Across a Chemistry Curriculum

Chloé Morin, *University of Minnesota-Rochester*

Juquila Contreras Vital, Vanessa Andrade, Destiny Regalia, Safa Sheikhibrahim, Cassidy Terrell

Biochemistry is an upper-division course that teaches topics using visual representations of systems of data, which can be a challenging way for students to learn. To lessen their cognitive load, students may find that improving visual literacy skills aids their understanding of biochemistry. There is little existing research that assesses how students interpret and store biochemical information and representations in their long-term memory. Previous studies performed with undergraduate general chemistry I students measured structural knowledge, or neural networks, of topics by asking students to assign the relatedness of chemistry-related words/phrases. Our study intends to analyze neural networks of a biochemistry visual literacy skill where undergraduate chemistry and biochemistry students to rank the relatedness of biochemical representations as an alternative to words/phrases. Specifically, this study assesses students' structural knowledge of the horizontal translation visual literacy skill, relating to the oxygen binding concept in comparison to the enzyme-substrate concept. We want to determine if there are pedagogical strategies and/or course instructional modalities that impact students' neural network development toward expert-like organization of the horizontal translation visual literacy skills. Preliminary analyses assess whether students are becoming more expert-like in correlation with their exposure to chemistry and biochemistry concepts. The data from student responses are analyzed in Pathfinder against an expert reference network to generate average degree and eccentricity values, path length correlation (PLC), and neighborhood similarity (NS) values, as well as patterns in organization/chunking. Degree values indicate the most branched nodes, while eccentricity values indicate the most central node in the neural network. PLC indicates how well each node is connected, and NS values are similarities in the grouping of concepts around the central node. Moreover, patterns in organization/chunking allow for similar groupings of nodes to be assessed. Through this study, we hope to improve curricular materials for biochemistry learning, in hopes that students will become more expert-like throughout their chemistry and biochemistry sequence. The outcome of this analysis may aid the improvement of curricular materials for optimal learning and retention of information.

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Abstract 2099

Easing Students (and Faculty!) into Big Data: Resources, Tips and Strategies

Walter Novak, *Wabash College*

Biochemistry is a data-heavy discipline; there are more than 230 M sequences in GenBank, 750 M organic compounds for virtual screening in ZINC, 200 M sequence-derived structural models in AlphaFold, and 180000 structures in the Protein Data Bank (PDB). Moreover, these repositories are rapidly changing as new data is added each year. Even analyzing a single biological structure can have an immense amount of information; for example, the PDB entry for the ribosome (4v5d) has almost 300 000 atoms, and that doesn't include hydrogen atoms. Techniques like next-generation sequencing (NGS) are commonplace in the biotechnology industry and can generate hundreds to thousands of sequencing reads per experiment, necessitating familiarity with processing big data. These are just a few examples of big data: data that is large, complex, and difficult to analyze without specialized software. As educators, it is important to develop future scientists who can access, analyze, and integrate big data to ask complex biochemical questions. Yet, due to its complexity, teaching students to work with big data is absent from many undergraduate biochemistry programs. Examples of working with big data in the biochemistry classroom, lab, or undergraduate research experience are presented, including virtual drug design and sequence and clustering analysis. Additional resources and ideas are shared to foster cutting edge, big data-driven experiences with students.

This work is supported by the Wabash College Haines Biochemistry Fund.

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Abstract 2120

Using ENCODE Project Data As Part Of A Course-Based Undergraduate Research Experience (CURE): Student Perspectives And Feedback

William Conrad, *Lake Forest College*

At Lake Forest College, molecular biology is taught as a third-year upper-level course. A central learning objective of this course is for students to independently formulate and test hypotheses by evaluating genome-wide epigenetic data. In this course-based undergraduate research project, students (1) select high confidence differentially methylated regions (DMRs) present in colorectal cancer samples (2) justify their selection based on literature review (3) predict the effect of DMR methylation on gene expression based on ENCODE project data, (4) predict if the DMR is methylated in HCT-116 colorectal cancer cells, and (5) test if the DMR is methylated in HCT-116s and demethylated following decitabine treatment. I will present the student learning outcomes for the course and align how students are assessed according to these outcomes. I will also present anonymous survey data of students' perceptions of the scientific method and their perceptions of themselves as researchers as they engage in research based on big data projects.

Funded by the Biochemistry and Molecular Biology program at Lake Forest College.

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Abstract 2138

The Genomics Education Partnership empowers faculty from a variety of institutions to successfully integrate genomics research experiences into undergraduate courses by providing flexible implementation strategies, ready-made curricula, a support network

Katie Sandlin, *The University of Alabama*

Anya Goodman, Catherine Reinke, Melanie Van Stry, Paula Croonquist, Wilson Leung, David Lopatto, Laura Reed

The Genomics Education Partnership (GEP; thegep.org) is a nationwide faculty-driven collaboration of more than 250 members working to ensure all undergraduates, regardless of their background and available resources, can participate in genomics research via Course-based Undergraduate Research Experiences (CUREs). Integration of research experiences and/or inquiry-based learning into a variety of courses, including molecular biology, genetics, biotechnology, research methods, or introductory biology, allows instructors to engage students with bioinformatics tools and new sequencing technologies, while also introducing or reinforcing fundamental concepts such as eukaryotic gene structure, regulation of gene expression, and molecular evolution. GEP research projects currently focus on structural gene annotations, and the annotation protocols are exclusively web-based. Since large amounts of sequencing data are publicly available and the compute infrastructure is maintained by the GEP staff, the need for costly local research infrastructure is eliminated. GEP students require only a computer and reliable internet connection to participate. Thus, GEP is a cost-effective strategy for institutions to increase the number of research experiences accessible to students. While engaging in novel research, GEP students gain marketable skills in critical thinking and big data analyses by comparing empirical (e.g., RNA-Seq data) and computational (e.g., gene predictions, sequence alignments) evidence to generate gene models. Since its founding in 2006, more than 1100 GEP students have contributed as co-authors on published scientific research papers. Recently, GEP began offering students a more proximal authorship opportunity by partnering with microPublication Biology to publish individual gene model reports. Members of the GEP teach at over 200 colleges and universities across the United States and Puerto Rico — 71% are Primarily Undergraduate Institutions (PUIs), 23% are Minority-Serving Institutions (MSIs), and 13% are Community Colleges (CCs). To understand how faculty across institutions utilize the GEP curriculum and assess the student outcomes of those differing implementation strategies, we used faculty reports submitted for each course along with student responses to pre- and post-course knowledge quizzes and surveys of student attitudes and affect. Comparisons of implementation types were performed across lower- and upper-division courses at 4-year universities and CCs. Preliminary data suggests GEP students at CCs and 4-year universities show similar cognitive and affective gains. Similar student outcomes irrespective of institution type are possible because of the support provided by a dedicated small core staff and a larger volunteer community of faculty. After initial training, GEP members continue to receive teaching and technical support, including professional development opportunities, throughout their tenure with the Partnership. GEP students can consult with Virtual Teaching Assistants for real-time support seven days a week. The GEP has created a supportive community of faculty and welcome new colleagues (no experience necessary!) eager for the challenge of bringing genomics research to all undergraduate students. Faculty interested in joining the GEP can contact us at thegep.org/contact.

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Abstract 2146

Using Kinetic Simulation Activities to Stimulate Knowledge Integration and Systems Thinking in an Undergraduate Metabolism Course

Eric Jones, *California Polytechnic State University*

Despite widespread adoption of active-learning pedagogies in STEM disciplines, the subject of metabolism is still generally taught in a passive (lecture and reading) format. It is challenging to devise active, engaging, and relevant activities for a metabolism course due to the abstract nature and sheer volume of the course material. In the past few years, computational simulations, using online platforms such as CellCollective, have shown promise in engaging students of metabolism and producing positive learning outcomes in accord with ASBMB's Core Concepts. What remains to be determined, however, is whether such computational active learning activities transform the manner in which students think—that is, whether they help students to view metabolism in a more expert-like manner. This poster summarizes the ongoing effort at Cal Poly, San Luis Obispo, to use kinetic simulation activities to stimulate 1) deeper understanding of metabolic flux and regulation, as measured using an existing assessment instrument, and 2) knowledge integration, or the ability to recognize and describe connections between topics in metabolism and general chemistry and biochemistry. The latter will be assessed using a concept-mapping task currently under development. Pilot studies were conducted using kinetic simulations of metabolic models in SBML format, implemented in COPASI to allow for manipulation of basic chemical parameters. Preliminary results show both encouraging potential for simulations to increase meaningful student engagement with the course material, and pitfalls to avoid for those interested in using metabolic simulations in the classroom.

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Abstract 2183

Leading the institution to meet the students' needs

Marina Holz, *New York Medical College*

In my talk, I will discuss my role as the dean of the Graduate School, and our efforts to address the needs of our graduate students in a changing educational landscape. Graduate students in the biomedical PhD and MS programs are conscious of the financial and opportunity costs of pursuing graduate degrees. Students seek graduate degrees in order to embark on a variety of careers outside of academia or as an academic enhancer in order to pursue training in professional fields like medicine, dentistry and pharmacy. Modern graduate education in the biomedical sciences has to be tailored to meet the students' needs and maximize professional outcomes. Specifically, I will focus on adjusting the admissions requirements to remove financial and structural barriers to admission, aligning the curricula with the career goals of the students, and creating linkage opportunities with other professional programs, such as medical and dental schools to optimize educational paths for graduate students.

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Abstract 2198

CRISPR for all: Production of a scalable CRISPR workflow for teaching and research

Christian Santiago, *Rollins College*

Sebastian Hernandez, Jay Pieczynski

CRISPR has been labelled as a "once in a generation" technology and is quickly changing the scope and pace of both research and teaching. Therefore, it has become imperative that undergraduate students be introduced to CRISPR-based methodologies before entering the scientific or health workforces. Two of the most often cited barriers to teaching CRISPR in the undergraduate classroom are lack of familiarity with the required techniques and the cost associated with teaching and using them. To this end, we have begun the development of a nominal, yet easily scalable CRISPR workflow for teaching and research. This framework focuses on utilizing common molecular biology equipment and techniques that can be found in most institutes of higher education, all while keeping reagent costs to a minimum. We have developed plasmids for production of CRISPR gRNAs in common lab *E. coli* strains, whereas these gRNAs can be easily purified for downstream usage either *in vitro* or *in vivo*. In addition, we have also streamlined protocols for purification and quality control of reagents to teach undergraduates essential molecular biology techniques, but again keep technical training and financial barriers to a minimum in order to make CRISPR accessible to all levels of education and research.

Rollins College Student Faculty Collaborative Research Program and the Stella Van Houten Fund.

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Abstract 2230

Making course-based undergraduate research experiences work for faculty too

Ryan Steed, *University of North Carolina Asheville*

Amanda Wolfe

Course-based undergraduate research experiences (CUREs) have well-documented benefits for student learning, retention, and academic success. However, the additional promise that the development and implementation of CUREs enhances faculty research productivity is less supported by the literature. We conducted a national survey of faculty at research institutions and primarily undergraduate institutions (PUIs), both public and private, who have implemented CUREs to determine how often data generated in CUREs leads to publication in a scientific journal and the challenges associated with bringing the project to publication. Unsurprisingly, we found that institution type, faculty rank, CURE level (lower or upper level course), and other factors affected the likelihood of publication. Additionally, successful publication usually required multiple semesters of CURE-generated data and significant follow-up by faculty and/or research students to improve the quality of student-generated data, complete supplemental experiments, and write manuscripts. With these insights, we sought to restructure our own upper-level biochemistry CURE at a public PUI with a focus on overcoming the barriers to generating high-quality, publishable research. Our goal is to provide students with an impactful research experience while also benefiting the research goals of the faculty involved.

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Abstract 2236

Creation of Virtual Affinity Groups to Provide Support to Women Faculty in STEM Across a Consortium of Public Liberal Arts Colleges

Wendy Pogozelski, *SUNY at Geneseo*

Karleen West, Sally Wasileski, Chavonda Mills, Josephine Rodriguez, Cara Margherio, Darci Demobroski

Introduction: Although recent decades have seen improvements in access and climate for women faculty, women remain underrepresented in STEM. At many primarily undergraduate institutions (PUIs), representation of women is particularly poor, with growth lagging behind that of research institutions. One barrier is isolation, especially at smaller institutions where women may find few colleagues who share their experience. Moreover, gendered inequities at these institutions can increase needs for mentoring and psychosocial support. These issues are compounded for women with intersectional identities.

Objective: Our initiative, funded by the NSF ADVANCE program, targets women in STEM at the PUIs of the Council of Public Liberal Arts Colleges (COPLAC). This consortium, comprised of 29 member institutions across the U.S. and Canada, has leaders that meet regularly, and has a structure for communication. We developed a two-tiered approach, one of which was aimed at training campus leaders to recognize and rectify issues of bias. The second approach, discussed here, was the creation of an inter-institutional support network for women STEM faculty. We created virtual "affinity groups" to generate a sustainable infrastructure designed to combat isolation, foster community, empowerment, collaboration, mentoring, and solutions for women in STEM at member institutions.

Methods: In 2020, our pilot program created nine Affinity Groups of 56 total participants, formed around themes that arose from an interest survey (ranging from area of specialization to intersectional identity to topics related to professional development). We recruited leaders/facilitators who facilitated monthly virtual meetings. We expanded the program in 2021 and then again in 2022 to ultimately involve 18 groups with 103 participants, representing a broad range of topic themes.

Results: Women largely continued participating in the groups over this three-year period, and membership grew. Assessment revealed a high level of satisfaction, and open-ended responses showed that participants found the groups to be highly beneficial. Success has required committed leaders and participants, and an emphasis on finding solutions rather than merely "venting." Additional benefits include participants receiving tenure, promotion, and submitting grant proposals, as well as reports of increased feelings of support. We note the broad applicability and sustainability of this kind of program and show how it can be adapted by other institutions and organizations.

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Abstract 2243

Otterbein ASBMB Student Chapter Outreach and Involvement

Olivia Miller, *Otterbein University*

John Tansey

The Otterbein ASBMB Student Chapter contributes to the development and growth of its members by providing outreach and scholarly activities. We meet at the beginning of each semester to plan our upcoming calendar. Some events are led by faculty, some by students, and others are led by an outside organization (such as an area science museum). The events can be categorized into academic events, professional events, and recreational events, but all have a biochemical or STEM theme. We host or participate in multiple outreach events each year. Last fall, our members joined with other campus STEM departments for 'Running on STEAM', an outreach event for a local middle school track team. At this event there are multiple tables for students to engage in a variety of scientific experiments ranging from isolating DNA from strawberries to making bouncy balls or examining brine shrimp under a microscope. The members practiced their communication skills by explaining the science in each experiment in terms the general public could understand. A second event we hosted this past year was a celebration of Mardi Gras. The campus dining hall served traditional spicy Cajun foods, and our Student Chapter produced a poster highlighting the spice capsaicin and other spices found in these dishes. At the end of the school year, members travel to our local science museum (COSI, the Center of Science and Industry) for the annual COSI Science Festival. The theme was 'stinky science' and members explained how the sense of smell worked and related stinky smells and their molecular shapes. This fall, members hosted a booth at our campus 'Teach-in for the Planet' event. The theme was based on our campus community garden and Chapter members discussed the biochemistry of honey and some of its properties. Other events focus on academic goals or professional development. We hold a picture day where students can have professional resume photos taken in lab coats to use for their LinkedIn and Handshake profiles. In addition, every semester we host an academic advising night allowing members to meet with faculty to discuss upcoming class schedules and internship opportunities. It also provides the chance for underclassman to meet upperclassmen and foster a mentoring relationship. Lastly, recreational events provide a time to destress from classes and engage in a different form of learning such as soap-making, painting, or terrarium construction. Each of these events has a biochemical theme and a flyer that explains different biochemical concepts related to the project. Our student chapter aims to give back to the community and support members throughout their academic journey.

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Abstract 2255

Community Partnership Can Drive Continuous STEM Exposure for Female-Identifying and Non-Binary Youth

Shyretha Brown, *Building Bridges, Inc.*

Jaclyn Carmichael, Margy LaFreniere, Aaron Price, TaAqua Campbell

The most engaging science, technology, engineering, and mathematics (STEM) outreach events are often those that are planned, integrated, and executed by community partners and stakeholders. When creating STEM outreach opportunities for middle school youth organizations, it is necessary to showcase STEM skills that translate to real careers all while providing a STEM experience with a STEM professional. The Museum of Science and Industry (MSI), Chicago is one of the largest science museums in the world and home to more than 400 000 square feet of hands-on exhibits designed to spark scientific inquiry and creativity. Building Bridges, Inc., a non-profit organization (EIN: 82-5445077), was born out of a desire to empower young Black girls by exposing them to STEM education and self-awareness strategies. Both STEM-based organizations partnered together to deliver the 1st Annual Youth Summit at MSI on Saturday, October 8th for middle school female-identifying and non-binary youth. This conference-style event was curated to engage middle school-aged youth in interactive, hands-on activities centered around subjects such as nature-based science, gaming, and health & wellness with STEM professionals. A total of 60 middle school female-identifying and non-binary youth and 13 chaperones attended this 5-hour event. Participants from three youth organizations were put into random groups and chaperoned to five 30-minute sessions. As a partnering STEM organization, Building Bridges, Inc. led five sessions titled "Love the Skin You're In" which exposed participants to the biology of skin, skin structure, importance of skin care, and innovation by making their very own sugar scrub to take home. 65% survey feedback was received from youth, and survey responses declared that 43% had never thought about STEM before interacting with Building Bridges, Inc. Youth (96%) are open to learning more about STEM by doing more hands-on activities with Building Bridges, Inc. Some youth (86%) could see themselves considering a career in STEM if they continued to interact with Building Bridges, Inc. Overall, this feedback suggests that middle school female-identifying and non-binary youth may benefit from continuous exposure to STEM activities and professionals through organized STEM outreach events from community partners and stakeholders.

All materials for the STEM session were funded by the Museum of Science and Industry.

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Abstract 2260

With graduation on the horizon, *ungrading* motivates learners to achieve their goals

Orla Hart, *Purdue University*

We condition students to attach grades to every piece of learning throughout most of their schooling. Unsurprisingly then, grades are the primary motivation for learning for most students. The problematic nature of this exclusively extrinsic, comparative, method in assessing success is clear when we consider professional careers and graduate work. The "ungrading" movement has gathered steam in the last couple of years, especially as educators found themselves rethinking assessment strategies during forced distance learning. Briefly, ungrading refers to an instructional approach that decenters, or even eliminates, grades, and instead focuses on providing detailed frequent feedback to students to help them achieve the course goals. Here, I describe an ungrading pedagogy applied to a Biochemistry Senior Seminar (capstone) course and the preliminary findings and student perceptions. It should be noted here that Purdue, like most institutions, requires that I assign mid-term and final grades to students. The assigned grades were mutually agreed on by students and me following a formal mid-term and end-of-term reflective self-evaluation. The course is focused on science communication, with one of the primary deliverables being a presentation of senior research projects at a departmental poster presentation. The structure of the learning environment was deliberately collaborative, beginning with a collective agreement to engage in this ungrading experiment, and students brainstorming specific individual and collective course goals. This initial involvement of students being able to define their own metrics of success proved to be a powerful motivator as the semester unfolded and the seniors had more demands on their time and attentions. Following each assignment, students scheduled individual or group evaluation/feedback meetings with me to discuss the assignment and their progress. Findings of note that emerged during the semester: >95% of students submitted their assignments on time, and 100% of students submitted assignments with reminders. 100% of students scheduled feedback meetings. I spent no time grading, and instead spent that time giving feedback to students about how they were doing and discussing how to better reach their goals. I got to know my students, and they got to know me. Students did not universally assign themselves As in the course, and only occasionally, I had to overwrite a student's grade (both raising and lowering) to one that I felt better reflected their engagement and progress. Interestingly, students presenting as female consistently gave themselves lower self-evaluations in 6 out of 8 categories than their male-presenting classmates, though the sample size is too small for any robust analysis at this point. While it is likely that ungrading will never be an appropriate pedagogy for most of the Biochemistry program courses, the current outcomes indicate that it is working well for this capstone course, and I will continue to implement it in future iterations of the course. This success may be due to the proximity to graduation, but feedback from students also indicates that the autonomy in defining what their own success looks like is a critical factor to their motivation. Additional data on self-perceptions of competence across gender and other dimensions of diversity will be analyzed as the sample size increases and may highlight areas that our department can focus on to increase equity across the undergraduate students.

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Abstract 2285

Developing a collaborative parasitology Course Undergraduate Research Experience (CURE) as part of a core biochemistry course sequence

Amy Greene, *Albright College*

Integrating Course Undergraduate Research Experiences (CUREs) into core courses sequences rather than as stand-alone research courses can be advantageous especially at small liberal arts colleges with limited course rotations. Starting in the spring of 2020, I designed a parasitology research CURE for the core biochemistry course sequence at Albright College, developing metabolism-focused labs to complement course content and scaffolding experimental design for a student-designed project. Our organism model is *Crithidia fasciculata*, a non-pathogenic trypanosome related to parasites causing African Sleeping Sickness, Chagas' disease, and Leishmaniasis. CURE students plan their own metabolic competition experiments, observing glucose metabolism via C-13 NMR, and purify parasite lipids for mass spectrometry identification. Finally, students develop novel research projects based on individual interests. The students write drafts of the student project article throughout the semester in pieces, ending the semester with a professional-style scientific article. In order to develop networking and collaboration skills, students interacted online with expert seminar speakers and parasitology CURE students at other institutions. CURE models are flexible to accommodate institutional and course needs while providing the benefit of student-led novel research and the development of collaboration and other process skills.

ACRE - Albright Creative Research Experience CURE author fellow ENCOUR Fellowship for Ethics and Responsible Conduct of Research (E/RCR) integration in Course-based Undergraduate Research Experiences.

103544, https://doi.org/10.1016/j.jbc.2023.103544

Abstract 2290

Process of Developing a Cultural Wellness Theme Enhancing Inclusion in STEM: a Community-Building Co-Mentoring (CoCo) Café Model

Beverly Smith-Keiling, *Univ of Minnesota*

Katrina Paleologos, Ellie Vraa

To develop inclusive connections at a large R1 university, a unique, diverse, community-building co-mentoring (CoCo Cafe) model program evolved. Our method was an ethnographic (based on grounded-theory), observational, and exploratory intervention to identify and address barriers in STEM education. Self-study within the CoCo Café community included over 30 participants spanning four cohorts predominantly recruited from our upper-undergraduate Biochemistry Lab course, graduate students, occasional faculty, and postdocs. As we engaged, we used the common term "we" to discuss our outcomes as a multi-ethnic, multigenerational team learning from each other in community co-mentoring. Our common motivations focused on research and academic development of new skills in the context of cultural wellness. We recognized knowledge is not always acquired through the academic institutional structure but also acquired through community and personal connections within the lived experience. Our grasp of cultural humility begins by recognizing historical trauma, distrust, and social inequalities that have pervaded throughout history within a EuroAmerican dominating culture. With the knowledge of self, our backgrounds within the context of past history and our present-day interactions, our program aimed to share knowledge that is culturally-relevant within an anti-deficit, cultural-wealth and wellness framework. To develop and employ our cultural wellness curricular theme, our process 1) listened to participants, identities, barriers. Through deeply reflective self-development, recognizing assets and overcoming barriers, we focused on our individual thought and deeper understanding of developing community culture supporting inclusion. We 2) developed community connections by engaging with a cultural wellness center (CWC) community partner, learning from their experiences and programming. As stakeholders in partnership with the CWC and its philosophy of healing through community, we reflected and took intentional steps to recognize and counter the underlying dominant structure embracing distinct lessons for cultural wealth and wellness in the curriculum, e.g. intersections of identity, assets from deeper root cultures, polychronic flexible time, distancing from individualistic 'bootstrap' mentality to collectivist. 3) We developed trusting relationships through student liaisons, co-funded cultural wellness sessions with Elders in the community, and 4) through a process of student reflection and feedback, we discussed and developed talking points for this curricular theme embedding it within our application-based curriculum. Thus, as participants engage in community-building practices supporting equity, we applied research and professional development

skills. Our results included 5) a process evaluation of the program through our program coordinator roles, 6) a developed program evaluation logic model to map outcomes as participants, 7) evaluated growth through our own program post-survey documenting that all 30/30 benefited. Only a few were lost-to-follow-up with only one not yet completing graduation. This focused, underlying cultural wellness curricular theme was practiced with diverse groups from other institutions and resulted in publication. Part of the challenge in addressing diversity, equity, and inclusion in education is to extend beyond the institutional academic system. The CoCo Café model begins doing so.

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Abstract 2293
Teaching in the time of post-COVID-19

Phillip Ortiz, *SUNY*

Carolyn Dehner

Although it can be argued as to whether the COVID-19 pandemic is completely behind us, it does seem that the worst may be over. Regardless, the effects still loom large, and colleges and universities are working to return to normalcy, educators are confronted with unprecedented challenges in student readiness such as insufficient learning, loss of cognitive sharpness, behavioral and psychological challenges, inattentiveness, loss of study and interpersonal skills, and so forth. These problems may be further exacerbated if the developmental windows for learning certain skills and skillsets closed during the pandemic. Building upon a series of manuscripts published in Biochemistry and Molecular Biology Education [BAMBEd] that were submitted in response to a March 2020 editorial titled, "Teaching in the time of COVID," which called for educators to share their strategies for addressing the immediate challenges in biochemistry and molecular biology education caused by the pandemic, as well as a similarly titled workshop we led at the 2022 ASBMB Annual Meeting in April 2022, we will present a number of innovative techniques that employ alternate teaching modalities (such as asynchronous, synchronous or hybrid remote instruction, HyFlex, etc.). In addition, we will address challenges that were often exacerbated by the 'digital divide' and other socioeconomic factors. These effects remain evident as we enter the post-pandemic period.

103546, https://doi.org/10.1016/j.jbc.2023.103546

Abstract 2318

Does Order Matter when Teaching Noncovalent Interactions: A Comparison of the Sequence of Explicit Instruction and Problem Solving

Paula Lemons, *University of Georgia*

Cheng-Wen He, Logan Fiorella

The objective of this study is to compare the impact of two instructional approaches on learning about noncovalent interactions: instruction-followed-by-problem-solving (I-PS) and problem-solving-followed-by instruction (PS-I). Prior research shows that both approaches support student learning, yet the specific learning outcomes vary. I-PS may be superior for learning procedural knowledge while PS-I may be superior for applying learning to new problems. The proposed rationale for this difference is that I-PS students efficiently learn how to solve a particular type of problem during the instructional phase, while PS-I students struggle to solve problems prior to explicit instruction but leverage the struggle to discover their knowledge gaps and the underlying concepts of a problem. We directly tested this idea among undergraduate students enrolled in an introductory biochemistry course for science majors at a large research university. Students (N = 138) in introductory biochemistry were randomly assigned to complete a lesson about noncovalent interactions in either the I-PS or the PS-I format. All students individually completed a problem-solving activity during a single class period. Outside of class, I-PS students watched an instructional video the day before the problem-solving activity, while PS-I students watched the instructional video the day after the activity. Three days after the problem-solving activity, all students completed a posttest, which included near-transfer problems that resembled the problem-solving activity and far-transfer problems that required application of the concepts from the lesson. We conducted statistical analyses to compare the I-PS and PS-I groups on each measure. This study was approved by the University of Georgia IRB (project PROJECT00003989), whose rules comply with the Declaration of Helsinki principles. Students in the I-PS treatment performed significantly better on the in-class problem-solving activity compared to PS-I students. We found no statistically significant differences between the performance of the I-PS and PS-I students on the near transfer posttest problems, although there is a trend in the data showing stronger performance for I-PS students. We are still analyzing the results from the far-transfer posttest problems. Our results show that the I-PS approach initially leads to more successful problem completion. This finding was expected based on prior research. Our data also show that neither the I-PS nor PS-I approach provides an advantage in near-transfer problem-solving performance days after learning. This may be because the near-transfer problems closely resembled the problems solved during the lesson, and it is possible that explicit instruction is beneficial for near-transfer problem solving regardless of whether it comes before or after problem solving. An important remaining question is whether either group was advantaged in far-transfer problem solving. The hypothesized benefit of PS-I is that it does a better job in helping students discover the underlying concepts of a problem and, thus, transferring learning. If, indeed, that is the case, we expect PS-I students will perform better on the far-transfer posttest problems. These findings can be leveraged to create biochemistry lessons that maximize students' ability to solve biochemistry problems and apply their knowledge to novel problems.

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Abstract 2343

Enhancing the Careers Exploration of Undergraduate Students at a Public, Hispanic Serving Institution: The ÉLITE Career Mentoring Program

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Tyrel Bryan

Mentoring is a transformative experience for improving students' confidence, develop transferable skills, and identify and achieve career goals in the STEM undergraduate setting. A successful and active mentoring program brings benefits to mentees, mentors, other faculty and the University. Increasing inclusion of underrepresented minority and first-generation students in mentored experiences both increases diversity in the life sciences community and prepares students for successful careers in these fields. ÉLITE Career Mentoring Program is a career advancement opportunity for a diverse population of students at a public, Hispanic Serving Institution (HSI). ÉLITE aims in connecting Biochemistry students with professionals in order to guide their career decision- making and develop a professional network. An intrinsic goal of the ÉLITE program is to support underrepresented and first-generation college students make the transition to jobs, professional and graduate school. Here, we provide mentorship through professional development seminars where experts give advice, professional inspiration, and insights about various career paths in biochemistry and other fields. We examined students' satisfaction, participation and engagement, as well as, we evaluated the program's appropriateness, outcomes and benefits from students' perspectives. Students were highly satisfied with the ÉLITE Career Mentoring Program, gaining academic and non-academic achievements. Students increased interactions with professional from different STEM fields, gained confidence in pursuing biomedical careers, improved their communication skills, and felt prepared for career exploration and flexibility.

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Abstract 2344

Threading Biotechnology through the Curriculum Enhances Student identification as a STEM Professional and Critical Thinking Skills

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Biotechnology utilizes many fields of study by its very nature, and benefits society by developing medicines, discovering disease pathways, and ensuring a stable food supply. Students need hands-on training to be effective in the biotechnology workforce. Students are trained in various aspects of biotechnology during their college coursework, however they may not connect the information. As a result, students are not aware of professional opportunities in biotechnology. Often first generation and demographically minoritized students (URM) do not gain access to research opportunities, a high-impact educational practice. Here we sought to broaden access and clearly link biotechnology curriculum to a theme that covers many different areas including health and disease, agriculture, genetic editing, ethical practices in biology, enzyme studies and the pharmaceutical industry. To accomplish this, a curriculum thread that promotes deep learning was created. Faculty from multiple disciplines developed related content to a linked theme with hands-on inquiry-based activities and laboratory research experiences. The explicitly linked material on the theme of polyphenol oxidase biotechnology was incorporated into freshman through upper division courses. Content was developed for use in lower division courses: Becoming a STEM Professional, Principles of Biology; and upper division: Genetics, Organic Chemistry, Cell Biology, and Biochemistry. Student assessment survey results were analyzed and indicate enhanced student outcomes. These include a stronger interest in biotechnology research in future careers (N = 142, 59% all students; 60% URM); a gain in identifying as a scientist from six response measures (N = 140–144, 80.1%, Std. Dev. 2.5 all students; N = 47–48 80.8, Std. Dev. 5.1 URM students); better critical thinking skills from four response measures (N = 142, 78.0%, 4.36 Std. Dev. all students; N = 48 80.7%, 4.6 Std. Dev. URM); and a discovery of the interdisciplinary nature of biotechnology versus previous knowledge. These results indicate that theme threaded content offered over multiple semesters is beneficial to critical thinking and fosters deep learning. Content modules are in preparation for distribution to faculty and the broader academic community with resources to facilitate implementation in other settings.

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Abstract 2363

Analysis of the Undergraduate Research Experiences through the Lens of Job Characteristics Theory: project CUREOS

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Undergraduate research experiences (UREs) are among the most impactful practices promoting student persistence, self-efficacy, and science identity in STEM careers. How do UREs promote these positive student outcomes? The role played by the structure of the work, or the way that students' activities are designed, in undergraduate research has not been characterized. We use Job Characteristics Theory (JCT) as a theoretical framework to characterize how the work design of UREs generates positive student outcomes in STEM fields. Based on JCT, core job characteristics (e.g., skill variety, task identity, task significance, autonomy, and feedback) affect work-related outcomes, including motivation, satisfaction and performance (Hackman, J. R. & Oldham, G. R., 1975). We are adapting existing survey items from the literature used to measure job characteristics to the undergraduate research context. We are using cognitive interviews to validate adapted survey items and to identify related outcomes of students' research experiences. For survey item validation (or revision), we use coding provided by multiple raters using three criteria, which Karabenick et al. (2007) defined as item interpretation congruency, coherent elaboration congruency, and answer choice congruency. We are especially interested in characterizing the work design and implementation of UREs during the academic year at undergraduate-focused institutions. UREs during the academic year pose unique challenges for students, including balancing the demands of research with coursework, employment, student clubs, sports, and family obligations. URE work design structure intentionally addressing these challenges has the potential to improve student outcomes. Based on the first round of interviews, we present emerging models for different work structures in the UREs. In the future, we will explore the relationships between structural facets of URE design and key student outcomes. One of our goals is to provide recommendations for improving the design of UREs, including course-based undergraduate research experiences, ultimately improving the experiences of students in pursuit of STEM degrees, for all students, and increasing the diversity of the STEM workforce. Project CUREOS (https://cureos.org) is looking for partners interested in department-level or program level assessment of undergraduate research, as well as individual students and faculty willing to share their experience by completing surveys and/or interviews.

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Abstract 2367

Integrating Authentic Biochemistry Research and Peer-Reviewed Scientific Literature into the High School Classroom

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Morgan D'Ausilio

Course-based undergraduate research experiences (CUREs) have been widely adopted at post-secondary institutions as a way to engage more students in authentic research. Several CUREs report measurable gains in student outcomes, including technical skills, content knowledge and persistence in science. In light of national initiatives to increase investigation and design in K-12 classrooms, educators are designing research experiences for students at increasingly early stages of their STEM education. However, there are many challenges to designing and implementing research experiences within a K-12 setting. At The Pingry School, we have adapted the Biochemistry Authentic Scientific Investigation Laboratory (BASIL) CURE to our upper level STEM curricula. The primary research objective of the BASIL CURE is to determine the enzymatic function of uncharacterized proteins using wet-lab and in silico investigation. We demonstrate that BASIL is sufficiently flexible to implement in a secondary school setting, either fully or in part. To facilitate planning within a high-school academic calendar, we have defined six "research phases" of BASIL as benchmarks for our students' progress. These phases serve as a framework for computational and biochemical analysis of enzyme function, while students gain experience in critical thinking and molecular biology techniques. Within each research phase, students demonstrate their competence by completing a set of defined "deliverables" in which they analyze the data they have collected in the form of a written report or presentation. To augment the research experience and to develop our students' scientific literacy, we have integrated regular journal club discussions into the research class curriculum. Students demonstrate a high level of engagement in this component of the course, and their comprehension of peer-reviewed articles is assessed both during and after journal club meetings. Adopting the BASIL curriculum as a part of our secondary school course design has facilitated communication with other educators in the BASIL consortium, a growing community that will prove an invaluable resource for K-12 educators seeking to establish or expand their research curriculum.

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Abstract 2388

Getting Excited: Evaluating the Impact of Enthusiasm on Science Communication Among Disadvantaged Groups

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Tyler Merrill, Orla Hart

For years, the Purdue University undergraduate chapter of the American Society for Biochemistry and Molecular Biology (PU-ASBMB) has been committed to making science education more accessible to members of the Greater Lafayette Community (GLC) through our programs and outreach events. PU-ASBMB has designed a service-learning outreach project which aims to improve accessibility, equity, and learning in the GLC. We intend to achieve this goal by investigating how science can be communicated most effectively to young (K-8) members, regardless of background. In our experience, communicating science traditionally can produce a mixed bag of results, which often includes a lack of engagement, retention, and organization. Rather than place the blame for this on 6-year-olds, it is worthwhile to examine how we can improve pedagogically. Specifically, we hypothesize that cultivating enthusiasm for science among younger groups is very important and correlates to students' continued interest in science. As such, it should be prioritized more often. Additionally, we believe this approach is especially important in effectively educating the youth of marginalized communities, who might not be reared in cultures/households which promote scientific enthusiasm. The results of this study, as well as the activity of the study itself, will serve to bring further accessibility, equity, and engagement for science-based educational opportunities to the GLC.

Methods: PU-ASBMB maintains a partnership with Hanna Community Center's after-school program, which includes over 20 K-6 students from 7 schools in low-income districts (www.hannacntr.org). Lesson plans are revised to focus on cultivating enthusiasm (eg. hands-on experiments, unprompted observations, comparing thoughts with other students), and data is collected in the form of simple surveys, qualitative observation, and basic interviews with students, staff, and volunteers. The data PU-ASBMB collected at Hanna Community Center in previous studies (presented at EB 2022) will serve as a baseline for evaluating the effectiveness of our outreach. Preliminary

Results: Limited qualitative data currently available suggests that prioritizing enthusiasm is effective in communicating science to youth, regardless of background. Interested students are more likely to engage with our activities and participate in learning. In short, students who are more engaged in science, will further pursue science.

Conclusions: Evaluating our own teaching approach allows PU-ASBMB to engage with our community in a more meaningful way, and the results of this study can be used by other groups to do the same. We believe a significant part of this study is the universality of its application. It is important for outreach groups to communicate as effectively as they can to their whole community, not just those with a background in/culture which promotes science. There is not much pedagogical research to support student groups in informal settings, which needs to change if universities are to be connected to their communities in genuinely meaningful ways. This presents a huge opportunity for research which will be conducive to promoting scientific accessibility, equity, and enthusiasm.

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Abstract 2400

The inaugural pd|hub Collection: a national resource for biomedical training programs seeking to enhance the career development of graduate students and postdocs

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Bill Lindstaedt, Elizabeth Genné-Bacon, Barbara Natalizio

Graduate students and postdocs pursue a wide variety of career paths–a reality that benefits the advancement of science and society. However, training programs have traditionally focused on preparing scientists for academic research, with minimal support for exploring the diversity of sectors and roles that scientists pursue. A lack of inclusive support for career development has been linked to the mental health crisis in graduate education and the loss of individuals from historically marginalized groups from science. Though calls to action have catalyzed efforts, too often educational interventions focus on exposure to career options and do not sufficiently acknowledge and support the complex developmental processes underlying career exploration. Through a new educational dissemination framework–the pd|hub Collections hosted by Professional Development Hub (pd|hub)–we have peer-reviewed and curated a set of nationally-sourced educational models (i.e., courses, workshops) that biomedical training programs can adapt to enhance their support of career exploration. The pd|hub Collections framework, through which this inaugural NIH-funded Collection ("Foundations of Career Exploration for PhD Scientists") is launching, was ideated by multi-stakeholder working groups initiated by ASBMB as a way to advance evidence-based practices in graduate and postdoctoral education. The framework facilitates the spread of evidence-based practices by a) curating concise, diverse collections of educational models for various professional development competencies, b) providing resources, training, and ongoing consultation to universities ("implementation sites") to facilitate efficient adaptation, implementation, evaluation, and evolution of the featured models, and c) incentivizing, recognizing, and supporting educators seeking to disseminate their work. In this session, participants will (a) learn about strategies to enhance equity and inclusivity in science while addressing the complexity of career exploration, (b) identify strengths and gaps in how their training programs support career exploration as a fundamental component of trainee professional development, and (c) learn about how to access resources of this pd|hub Collection to efficiently implement and evaluate evidence-based practice within their programs. We will also preview next steps for pd|hub, including development of future pd|hub Collections on other professional development competencies.

ASBMB, Burroughs Wellcome Fund, and NSF (1848789) funding contributed to ideation of the pd|hub Collections framework; the inaugural pd|hub Collection presented here is funded by the National Institute of General Medical Sciences of the NIH (R25GM139076). One of the models in this pd|hub Collection was developed with funding from NSF (1735210).

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Abstract 2402

Exploring Biodiversity in Metagenomics CUREs for General Biology

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Consuella Davis, Diane Sklensky

Course-based undergraduate research experiences (CUREs) provide opportunities for all students to engage in scientific discovery. At Lane College, the Biology Department's goal is to increase student engagement in General Biology by incorporating CUREs in the General Biology laboratory courses. In the project described here, students isolate and characterize antibiotic resistant bacteria from soil samples. The newest iteration of this project incorporates metagenomics with next-generation sequencing. Specifically, students use 16S rDNA barcoding and sequencing with the Nanopore MinION to identify the genus of the isolated bacteria using the Epi2Me 16S fastq algorithm. Students then explore the phylogenetic relationships between their isolated bacterial strains. They can also use whole genome sequencing and the Epi2Me antibiotic resistance algorithm to explore possible mechanisms of resistance. Faculty are expanding the metagenomics projects to explore biodiversity of campus plant and fungal species. Together, these projects emphasize foundational concepts covered in General Biology II, including biotechnology, evolutionary relationships, and ecology.

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Abstract 2403

Structure, Function and Regulation of Human Malate Dehydrogenase: A Platform to Drive Interesting and Sustainable CUREs

Joseph Provost, *University of San Diego*

Integrating research into the classroom environment is an influential pedagogical tool to support student learning, increase retention of STEM students and help students identify as scientists. The evolution of course-based undergraduate research experiences (CUREs) has grown from individual faculty incorporating their research into the teaching laboratory to well-supported systems to sustain faculty engagement in CUREs. To support the growth of protein-centric biochemistry-related CUREs, we created and grew a community of enthusiastic faculty to develop and adopt malate dehydrogenase (MDH) as a CURE focal point. Central to this protein-centric CUREs community is a fantastic resource of over 20 MDH His-tagged clones and hundreds of site-directed MDH mutants, protocols, and other supporting resources. The science of one set of MDH clones, human cytosolic (hMDH1) and mitochondria (hMDH2), will be highlighted in this presentation. Specifically, we will introduce the necessary background of human MDH and provide a review of the structural, kinetic, and regulation of MDH. Examples include the expression of cytosolic splice variants and our understanding of how the amino-terminal truncations impact MDH function. The many pathways of cytosolic and mitochondrial MDH that require the enzyme's directionality switch are highlighted to emphasize the unique properties of the enzyme in metabolism. The background of the metabolites known to regulate the activity of MDH will also be presented. Combined, how MDH is expressed and involved in various metabolic pathways will provide evidence highlighting the role of MDH in disorders from cancer to Parkinson's disease, diabetes, and metabolic disorder. Both isoforms of MDH are post-translationally modified by phosphorylation, methylation, and acetylation, yet little is known about the impact of these modifications on MDH activity. In addition, the potential protein-protein interacting partners with both MDH isoforms will be presented. Finally, the possible workflow to use this information will be shared with the MDH CURE community and those interested in joining.

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Abstract 2408

A New CURE in Molecular Biology: Testing a Novel Pyrrolidine Compound to Determine Mechanism of Action in an Upper-Division Molecular Biology Course

Sarah Mordan-Mccombs, *DePauw University*

The COVID-19 pandemic shut down forced introductory biology and chemistry laboratory courses online at DePauw University from March 2020-June 2021, leaving multiple classes of students without the opportunity to learn basic laboratory skills that are essential for the molecular biology laboratory. In an effort to provide students with both basic laboratory skills and advanced molecular biology skills, a new course-based undergraduate research experience (CURE) was developed for the 2022–23 academic year. In collaboration with Dr. Jeff Hansen in the Chemistry and Biochemistry department, novel compounds with potential anti-tumor properties were identified. The CURE in Molecular Biology was designed to have students use *Saccharomyces cerevisiae* as a model system to evaluate possible cellular pathways affected by the compound, including: cytoskeleton and cell migration, nucleotide biosynthesis, glucose metabolism, apoptosis, and cell cycle regulation. Students learned techniques DNA isolation and PCR, transformation, RNA isolation, cDNA synthesis, qPCR, and Western Blotting, while contributing to an active research project. At the conclusion of the project, students were surveyed about their comfort with molecular techniques and data analysis.

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Abstract 2422

Antibiotics from Ocean Plastic: Framework for a CURE

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Course-Based Undergraduate Research (CURE) is considered a high impact education practice that provides students with a research project of which they take ownership. While CURE topics can vary, students can be more engaged in topics with relevance to their lives. Plastic pollution of the environment is a global environmental concern. Microbes living in and on plastic debris constitute a novel ecosystem called the "plastisphere." These microbes undergo several steps of succession, eventually forming mature biofilms with a rich variety of organisms and functional activities. Most attention has been focused on plastic degradation and antibiotic resistance in the plastisphere. As an environment with resources and competition, similar to soil, we decided to explore the potential of the plastisphere to be a source of novel antibiotics. Our objective was to adopt the Tiny Earth framework, a crowdsourced approach to isolate antibiotic producing bacteria from the soil (https://tinyearth.wisc.edu/) to ocean plastic samples and explore if antibiotic producing bacteria could be obtained. Two types of floating plastic (high and low density polyethylene) were incubated 5 m deep in water near Scripps Pier in La Jolla, CA. After 90 days, plastics were retrieved and swabbed on Marine Agar and Tryptic Soy Agar. Resulting colonies were transferred to "library plates" and tested against selected Gram positive and negative bacterial strains using the spread-patch technique. Bacterial targets included safe ESKAPE relatives and antibiotic resistance strains from the CDC AR Bank# 0637. Producers were subsequently isolated and characterized by 16S metabarcoding. Sequencing results were characterized with the BLAST database and analyzed DNA Subway (dnasubway.cyverse.org). For organic extraction, both solid media and supernatant were used. Ethylacetate was the solvent used for solid media, and sequential heptane/ethylacetate extraction for supernatants. The resulting organic fractions were removed and evaporated. Solids were resuspended in methanol to a known concentration (1–10 mg/mL). Organic extracts were added to 96-well flat bottomed plate at different dilutions, and let dry. Bacterial test strains were added to the wells, and OD600 nm was measured after 18–24 hours. We describe the presence of five antibiotic producing bacteria isolated from the ocean plastic. Per 16S rRNA sequencing, HB1 is a Gram positive Bacillus. The rest are Gram negative rods, either Phaeobacter (H6), Cobetia (A5) or Vibrio (A2, AP22). Spread/patch testing showed A5 and H6 to have the broadest range of activity against both Gram positive (*B. subtilis*, *S. aureus*, *E. raffinosus*) and Gram negative (*E. coli*, *A. bayley*, *C. salexigens*) strains. The effect or organic fractions was more limited, with hexane fractions of A5 showing the best results towards antibiotic-resistant *Citrobacter freundii* and *Enterococcus faecalis*. In summary, marine microbes attached

to ocean plastic were revealed as a potential source for antibiotics effective against resistant bacteria. Further chemical and genetic characterization of the producers will reveal the exact nature of the antibiotic activity. Considering the public interest in plastic pollution, this adaptation can be used as an engaging course-based undergraduate research.

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Abstract 2427

Implementing DNA Barcoding and Metabarcoding for Course-Based Undergraduate Research Experiences

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Ray Enke, Jeremy Seto, George Ude, David Micklos

Early exposure to undergraduate research increases student retention and graduation rates in STEM. Educators rising to this challenge have incorporated course based undergraduate research experiences (CUREs) into large-enrollment freshman and sophomore STEM courses. The DNA barcoding and metabarcoding infrastructure developed by Cold Spring Harbor Laboratory's DNA Learning Center (DNALC) leverages modular CURE activities ready for implementation, as exemplified by the first year DNA barcoding CUREs at James Madison University (JMU). Our project is measuring impact of barcoding and metabarcoding CUREs that aim to meet faculty needs as they aim to enrich STEM education. The DNALC, JMU, Bowie State University, and New York City College of Technology have developed, disseminated, and evaluated these CUREs. We have trained, evaluate, and support educators from across the country as they implement the modules. To reduce barriers to implementation, the biochemistry and bioinformatics for barcoding and metabarcoding have been improved. Improvements including a new, faster DNA isolation method for DNA barcoding, an at-home DNA isolation method developed during the pandemic, streamlined biochemistry for metabarcoding, and ongoing improvements to online barcoding and metabarcoding bioinformatic pipelines will be presented. Three DNA barcoding workshops trained 35 university faculty, 26 college faculty, and 4 high school teachers. Meanwhile, two metabarcoding workshops have trained 47 university faculty, 27 college faculty, and 2 high school teachers. Two additional workshops at BSU introduced the CUREs (35) and trained faculty in metabarcoding (7). With extended support, including support with reagents, sequencing, pedagogy, and the biochemistry and bioinformatics supplied by project co-PIs and program participants acting as mentors, many faculty are implementing DNA barcoding and metabarcoding CUREs at their institutions. Participation in barcoding and metabarcoding CURES both had significant impacts on students' self reported knowledge of these approaches and attitudes towards science. Over half also felt they were capable of going further in science and were more interested in technology, bioinformatics, and studying biology. Broader Impacts: The program positively affects student attitudes of science, promising to increase the number of people pursuing careers in STEM. Students also contribute to biodiversity research, identifying and tracking species. The program also promises to increase the diversity of students pursuing STEM. Toward this goal, 28% of faculty trained in DNA barcoding and 20% of faculty trained in metabarcoding were underrepresented minorities. As hoped, faculty reached a large proportion of URM students, with 38% of

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students identifying as URM in survey responses. Coauthors Bruce Nash, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; Oliver Hyman, James Madison University, Harrisonburg, VA; Ray Enke, James Madison University, Harrisonburg, VA; George Ude, Bowie State University, Bowie, MD; Jeremy Seto, New York City College of Technology, New York, NY.

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Abstract 2429

Take a Deep Breath and Say Goodbye to Hemoglobin? Alternative Strategies for Teaching Protein-Ligand Concepts in Undergraduate Biochemistry

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Hemoglobin (Hb) and myoglobin (Mb) are hallmarks of undergraduate biochemistry curricula as they are used as the first exemplars of protein structure and function, and their associated and emergent properties such as affinity, allostery, cooperativity, as well as the effects of mutations on structure and function. Accompanying these are the mathematical models and graphs that describe binding and allostery. Yet few have questioned the roles of Hb and Mb as the first and key examples that students encounter when studying protein function linked to structure. In many ways, they are poor examples. Most ligands for proteins are not poorly soluble gasses like O2 and CO2. The axes labels on binding curve graphs are YO2 vs pO2, not those on the canonical protein-ligand binding graphs of fraction bound (r or Y) vs ligand concentration (molar units). In addition, Hb and Mb bind their major ligand, O2, through a coordinate or dative covalent bond, not classical noncovalent interactions such as salt bridges, hydrogen bonds, etc. In addition, O2 binds to a Fe2+ ion in a heme cofactor, not a typical binding site formed by geometrically localized sidechain and backbone atoms. Hemoglobin and myoglobin are also covered in many undergraduate biology courses, making their study somewhat redundant in biochemistry courses, which might discourage a more in depth learning of a topic they think they understand from previous coursework. Collectively, these factors could easily confuse students and prevent them from applying their learning gains to the majority of protein:ligand interactions. Given these confounding issues, we suggest other protein examples for study. Additionally we present an alternative learning sequence that satisfies the learning goals and objectives for protein-ligand interactions. This sequence was deployed in an undergraduate Biochemistry I course and we offer comparative student assessment analysis between the traditional and alternative protein-ligand sequences. We also compare student data measuring the neural network organization visual literacy skills related to this concept between the two learning sequences. Our goal is to offer educators an alternative strategy for teaching that offers students the opportunity to better conceptualize protein-ligand interactions, while still meeting the learning goals and objectives laid out by the American Society for Biochemistry and Molecular Biology.

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Abstract 2443

Incorporation of Learning Assistants enhances students' sense of belonging in introductory science courses

Katherine Friedman, *Vanderbilt University*

Katherine Clements, Cristina Zepeda, Alexandra Grace, Allison Leich Hilbun, Thomas Clements, Heather Johnson, Jessica Watkins, Cynthia Brame

The science education system profoundly impacts who becomes a scientist. The current system works less effectively for some groups (e.g., women; Black, Hispanic, and American Indian students; first-generation students) than others (e.g., men; white, Asian American students; continuing generation students), resulting in a limited scientific community that does not reflect the larger U.S. population or its values. Efforts to increase equity often focus on improving students' experiences in gateway science courses. These efforts reveal that engaging students in active learning improves student performance and reduces performance gaps between underrepresented and well-represented students. Other lines of investigation show that promoting students' sense of social belonging can reduce disparities in student outcomes. We hypothesize that the Learning Assistant (LA) model, developed in 2001 at the University of Colorado, has potential to increase equity in science education through both mechanisms. LAs are undergraduates who, having previously succeeded in a course, now work with instructors to facilitate active learning in the classroom. LAs meet weekly with the instructor to plan and refine learning activities that address student needs. In addition to receiving a stipend, LAs enroll in a pedagogy course that teaches strategies to support students' sense of belonging and to facilitate active learning approaches used in LA-supported courses. In prior work (CBE-LSE 21, 2022), we found that students in sections of an introductory biology course with LAs reported significantly higher sense of belonging over two semesters than students in sections without LAs. Further, students described five ways in which working with LAs in this course increased their sense of belonging in the larger STEM field: by reducing their sense of isolation; providing inspirational role models; helping them understand the STEM educational system; increasing engagement and confidence in their STEM-related knowledge and skills; and providing accessible and approachable sources of support. Here, we expand that work in two ways. First, we extend our investigation to General Chemistry. General Chemistry plays a particularly important role in the introductory science curriculum, serving as an entry point for many students' science paths. It is therefore a particularly important course in which to understand and support belonging. Second, we ask whether the incorporation of LAs benefits students across demographic groups. As a whole, students in introductory chemistry with LAs report significantly greater sense of belonging than students in sections without LAs, confirming our previous work. Furthermore, the ways in which LAs contribute to belonging are consistent across discipline, course, and instructor. In courses both with and without LAs, men reported higher belonging than women; racially and ethnically well-represented students higher than underrepresented students; and continuing generation students higher than first generation students. While the presence of LAs did not appreciably close these gaps, the sense of belonging reported by each underserved group was greater when the LAs were present than the baseline level reported by the corresponding well-served group without LAs. These results suggest that the incorporation of LAs in gateway science courses, while benefiting all students, has an important impact on the sense of belonging for our underserved students.

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Abstract 2453

A CRISPR CURE to simultaneously engage undergraduate students and K-12 educators

Tara Phelps-Durr, *Fort Hays State University*

Course-based Undergraduate Research Experiences (CUREs) broaden participation in undergraduate research by bringing research into the courses students need to graduate. Numerous studies have shown that students engaged in CUREs gain confidence in their own abilities and are more likely to pursue advanced STEM degrees. Fort Hays State University is a public, comprehensive institution located in rural western Kansas. Like most rural areas, there is a severe shortage of health professionals in western Kansas. Many FHSU biology majors begin their degree in the hopes of pursuing health careers but have little to no knowledge of biotechnology techniques such as CRISPR that are revolutionizing medicine. To prepare our undergraduates for careers in healthcare, we are developing a molecular biology course that will introduce students to CRISPR by engaging them in an authentic research project. In addition, the course will be offered simultaneously at a graduate level for K-12 educators. To earn graduate credit, the students (K-12 educators) will design CRISPR lesson plans and assessments they can implement in their classrooms. The basic structure of the CRISPR CURE has been piloted in a sophomore level genetics course. Through a twelve-week series of lab activities the students first learn about CRISPR technology and its applications. The students then 1) learn the basics of gene regulation and plant development, 2) computationally model the three-dimensional structure of regulatory proteins required for plant development, 3) design and carry out CRISPR experiments to create mutations in genes encoding the regulatory proteins, and 4) write a lab report or present a poster describing their results. The students reflect on their semester long research experience and provide a written description of the knowledge and laboratory skills they learned as well as how the knowledge and skills will be used in their future careers. The K-12 educators enrolled for graduate credit will be asked to report back the effectiveness of the CRISPR lessons they designed while enrolled in the course. Here we present the course and CURE design.

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Abstract 2490

Overview: Bias In-Bias Out in Data Science

Dr. Allison C. Augustus-Wallace, *LSUHSC-NO, School of Medicine, Office of Diversity & Community Engagement*

Implicit bias, also referred to as unconscious bias, plays a significant role in all aspects of our lives, including with respect to our judgement and decision-making processes. Inappropriately, implicit bias often yields discrimination, injustice, microaggressive behavior, and/or racism, which must be recognized and dismantled to prevent the lessening/loss of access to resources, such as quality education and equitable healthcare. Moreover, throughout our history, it has often been the interpretation of biomedical/genetic research that has served as the basis of the concept of "race" and its implications which have also shaped our lives, in respect to policies, practices, as well as the work that we perform/conduct, including biomedical research. Furthermore, as we are positioned at the cusp of wide-staged implementation of the use of genetics to create artificial intelligence platforms, such as facial recognition, to recognize disease, it is extremely important that we discuss implicit bias in biomedical research, as not to create additional health disparities. Yet, as biomedical research has and continues to serve as the basis of medical treatment, we as researchers have not necessarily addressed nor examined the contribution of biomedical research to the creation of health disparities, especially those identified in historically-excluded populations of Americans. As biochemists, geneticists/biomedical researchers, the time has come to examine the impact of implicit bias on biomedical research with respect to the study of diseases such as diabetes, metabolic syndrome, and mental health, to mitigate poor health outcomes and thereby health disparities, especially in historically-excluded, marginalized communities, which have been affected most detrimentally. Therefore, to set the stage of our 2023 ASBMB MAC Symposium, this work will provide the historical overview of biomedical research, including experimental design and data interpretation, which has contributed to health disparities thus far, to develop mechanisms to mitigate these circumstances for the future.

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Abstract 2499

Exploring the Effects of Psychosocial Factors on STEM Persistence at an HBCU through a S-STEM Scholarship Program

Candace Carter, *Lane College*

Melanie Van Stry

Lane College, founded in 1882 by former slave Bishop Isaac Lane, has strong history of educating underserved, minority students through student-center approaches. The Lane College S-STEM program aims to increase the number of low-income students entering STEM fields by providing scholarships, co-curriculum and high impact program activities to support Lane College students majoring in biology, chemistry, computer science, mathematics, or physics. These activities include development and implementation of new first-year STEM courses, including CHE 110 Scientific Literacy and Critical Problem Solving and PHY 111 Galileo and the Church. We have successfully recruited 3 cohorts of S-STEM scholars who have participated in several project activities including bi-weekly cohort meetings, undergraduate research projects, community outreach, internships, mentoring, and professional development. For the research project, we are exploring the development of non-cognitive factors known to impact student persistence in STEM (Grit, Self-efficacy, Growth Mindset, etc.) as S-STEM scholars matriculate through the program. Our initial measures of Grit and Growth Mindset for cohort 1 do not show a significant difference between S-STEM scholars and the control group of academically talented Power of Potential scholars. However, we have experienced low student participation in surveys during the period of remote instruction in response to the COVID-19 pandemic. For cohort 2, we measured self-efficacy for students in cohort 2 using the self-efficacy formative questionnaire developed by Gaumer Erickson and colleagues. Our data for the five students cohort 2 who completed the survey show an average score of 80.8% for focus, 60.8% for steps, and overall 72.5%. These initial data suggest that the students will benefit from targeted instruction aimed at developing growth mindset and self-efficacy.

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Abstract 2514

Getting Started with Coding in the Classroom

Paul Craig, *Rochester Institute of Technology*

Nichole Orench-Rivera

Coding skills are becoming more important for career development for students and faculty. Coding in Python and R are important skills that equip users to engage in more sophisticated data analysis and enable them to pursue new career opportunities. If you are interested, but you and your students have never studied computer programming, you probably have questions like these: Where do we start? What resources do we need? How much will it cost? Are there existing lessons and activities? Is there a community we can join for shared learning? In our presentation, we will describe our paths (we are both consider ourselves novice coders), provide suggestions for online resources and workshops for training and point to links for many open source resources for learning. We will also describe two very user-friendly coding environments: Jupyter notebooks and Google Colab, with links to existing notebooks that access resources that we all use in our teaching and research: NCBI, PDB, OMIM, UniProt and PubMed.

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Abstract 2524

The Conundrum of Race as a Biased Variable in Medicine

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Bias Medicine

Structural biases, thru the lens of race, have been embedded into our principles, philosophies and frameworks for healthcare delivery, and clinical decision making in congruence with the sociopolitical evolution of racial classifications in the United States. The downstream effect has been the striking perpetuation of disparities in health outcomes, as influenced by the ways race is navigated in biomedical research, implementation of treatment protocols and the characterization of patient populations epidemiologically. One of the starkest examples is the establishment of white or Caucasian racial identity as the sentinel reference standard for evaluating disease outcomes. In many respects, just as we describe with biases in personal behavior, these structural biases in healthcare delivery serve as blind spots which have been rooted in our systems of learning in academic medicine. This session will explore specific examples of how disparities in clinical practice have been influenced by structurally biased blind spots which are not rooted in sound science, and examine ways in which they have contributed to harmful disparities in health outcomes. We will explore the need to more critically consider the influence of ancestry in a rational way which dismantles race based and centered models of delivering medical care.

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Abstract 2535

Leading the Future of STEM by Centering Equity and Inclusion

April Dukes, *University of Pittsburgh*

It is the responsibility of STEM faculty, staff, administrators, and discipline leaders to create inclusive environments that support and retain diverse students, researchers, and colleagues. Despite tremendous efforts to increase the diversity of students and faculty in higher education STEM fields, individuals with underrepresented identities continue to face barriers to entry and success, with many choosing to switch degrees or pursue fields other than STEM. How can we make progress in equity and inclusion, where other efforts have failed? In this talk, we will introduce the Inclusive Professional Framework for Faculty (IPF:Faculty), which has been developed by the NSF INCLUDES Aspire Alliance. The IPF:Faculty includes attitudes, knowledge, and skills that can empower current and future STEM faculty and leaders in their efforts to be more inclusive in their interactions with others. We will also examine how departments and institutions can similarly apply the Inclusive Professional Framework for Societies (IPF:Societies) to equip change leaders within disciplines and institutions. Fostering a true, multi-layered welcoming environment for students, faculty, staff, and administration is necessary to meet the needs for broadening participation in STEM and is necessary to drive meaningful and lasting change towards equity and inclusion in STEM.

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Abstract 2539

Understanding the Types of Chemistry Representations used in Research Studies and Interventions Articles Based on the Representational Competence Framework

Nabiha Atiquzzaman, *Nova Southeastern University*

Rebecca Boadu, Adriana Corrales, Lyneisha Ward, Molly Atkinson, Maia Popova, Zahilyn Roche Allred

Since its introduction, the Representational Competence Framework by Kozma and Russell has captured the attention of many researchers and practitioners in science education. The framework suggests that individuals who have developed representational competence possess a set of skills and practices that representational competence possesses a set of skills and practices which allow them to reflectively use representations, singly or together, to think about, communicate and act on chemical phenomena. Furthermore, developing representations has been suggested to be essential for developing expertise in subjects like Chemistry. Thus, as part of a large collaborative project among three universities, our team has been reviewing the literature on the use and incorporation of the Representational Competence Framework within undergraduate chemistry education research and interventions. As part of the larger study, our team is characterizing the types of representations used as part of our literature sample of over 200 peer-reviewed articles. Our analysis includes identifying the number of representations used as part of the scholarly work, the type of representations (Lewis structure, potential energy diagram, etc.), and the classification of representation as symbolic, sub-microscopic/particulate, or macroscopic guided by Chemistry's Triplet (I.e., Johnstone's Triplet). The review holds implications for chemistry instruction, which could give insight into how the framework has been used with the chemistry education community and potential areas where more work is warranted. Preliminary results from this project will be presented at the presentation.

Abstract acceptance will qualify for PDG Grant provided by Nova Southeastern University.

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Abstract 2561

Strengthening the Leadership Capacity of HBCUs through the Implementation of a World-Class Leadership Development Program to Broaden Participation in STEM

Margaret Kanipes, *North Carolina A&T State University*

Comfort Okpala

The Center for the Advancement of STEM Leadership (CASL) serves as the nation's premier intellectual and scholarship-generating resource for examining and determining the kind of leadership that broadens the participation of African Americans in STEM and contributes to the development of a next-generation of leaders. In this presentation, we will highlight the CASL Leadership Fellows Program (LFP), a one-year professional development opportunity, that has been organized around three residencies consisting of guest presentations from HBCUs and higher education leaders, live webinars throughout the year, writing assignments, case studies, and assigned readings. The Leadership Fellows Program (LFP) curriculum integrates the cognitive, affective and behavioral domains of leadership through an HBCU lens to improve the effectiveness of the participants as emerging leaders who will broaden participation in STEM. Since 2019, the LFP has engaged STEM leaders at over 25 HBCUs and has been offered in person and virtually due to the pandemic. As a result of the LFP, our data has shown that the fellows have 1) an increased understanding of their leadership styles and strengths, 2) an increased readiness to engage in leadership activities to broaden participation in STEM on their campuses, and 3) an increased readiness to be engaged in the STEM reform community. Our outcomes demonstrate the importance of an intentional leadership development program like the CASL LFP which employs a multi-faceted approach to empower STEM HBCU leaders to broaden participation in STEM.

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ASBMB

Abstract 2577

Being Human in STEM: coupling curiosity with humility to shape a more equitable and inclusive STEM landscape

Sheila Jaswal, *Amherst College*

Since the murder of George Floyd, faculty, staff and administrators at institutions of higher education and participants in the scientific enterprise across disciplines and organizations have demonstrated a remarkable willingness to confront racism and other forms of systemic oppression in our spaces. As educators, we know that for individuals to learn something so that they feel ownership of the material, and can independently apply their knowledge, they need to be stretched beyond their sphere of expertise and comfort through an authentic and challenging learning experience. As scientists, when our research takes us into unfamiliar territory and we are challenged to read the literature and learn from experts in a new field, we relish such opportunities to indulge our curiosity. However, it can be much harder to apply our curiosity and embrace humility with regard to the unfamiliar terrain of identity, bias, privilege and systems of oppression, particularly while navigating dynamics and relationships with students. Furthermore, Human Resources-style training programs in diversity, equity and inclusion (DEI) can feel artificial, short and difficult to translate into pedagogical practice. Here I describe the Being Human in STEM (HSTEM) course model, which I argue provides an innovative approach for expanding critical consciousness about equity & inclusion among faculty that leads to their activation as accomplices in doing racial equity work and shifting institutional STEM culture. In HSTEM courses, faculty and students read the academic literature on structural racism in STEM and academia, other knowledge systems, and efforts to reform STEM while reflecting on their own identity, positionality and privilege, and learning about each others' lived experiences. Participating faculty experience being co-learners who are not experts, practicing humility, making mistakes and making amends, and sitting with discomfort. Faculty witness how students describe and reflect on their STEM experiences, using their new vocabulary and frameworks to put them in the context of institutional and societal structures, rather than their own individual deficits. In the students' culminating campus project proposals, which are presented at an annual all-campus salon, all faculty are confronted with descriptions of equity issues in their own STEM community that are grounded in the academic literature, and are offered creative evidence-based ideas for interventions that are informed by the collective student experiences. Thus, HSTEM is a context conducive to the cultivation of cultural humility and its extension into our practice of science in the classroom, lab and beyond.

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Abstract 2661

Participants Gain Significant Professional Skills and Communication Confidence through the Art of Science Communication Course: A Retrospective Survey Study

Christina Swords, *University of Wisconsin - Madison*

Nicole Woitowich, Jerlym Porter, Amy Hawkins, Edwin Li, Melissa Rowland-Goldsmith, Matt Koci, John Tansey

Public understanding of science is necessary to tackle modern challenges and to positively impact global communities; in a world of misinformation, improperly conveyed or inaccessible science can fuel fear and mistrust. Scientists can contribute to the positive promotion of science and its role in society through effective communication. However, formal training in science communication remains limited in higher education, tending to be viewed as extra-curricular rather than part of core learning objectives. To address this, the ASBMB Science Outreach and Communication Committee (SOCC) developed the Art of Science Communication (ASC) course in 2015, to provide broadly accessible science communication training to scientists at all career stages. The aim of this current survey study was to assess how the course impacted participants' science communication behaviors, their confidence engaging with non-experts, and other benefits to their professional development. Over 300 past ASC participants completed a 36-item survey instrument that assessed participants' engagement with science outreach and communication efforts. We found that participants engaged with non-expert audiences more frequently after taking the ASC course. Prior to the ASC, 51% ($n = 86$) of respondents indicated that they engage in science communication with non-experts sometimes (5–20 hours/year) or often (21+ hours/year). Following the course, this proportion significantly increased to 77% ($n = 128$) of respondents ($p < 0.0001$). Participants were also more confident in their communication skills after taking the course (Mdn = 8, M = 7.8 SD = 0.98 vs. Mdn = 5, M = 5.4 SD = 1.57, $p < 0.0001$). Likewise, participants reported benefits to their professional development. Trainees found their enhanced communication skills to be integral in their applications for graduate school or employment; whereas faculty found value in using these skills for career advancement or promotional purposes. Qualitative results echoed the quantitative analyses where themes of increased confidence, professional development, and audience engagement emerged from optional open-ended responses. These results suggest that the Art of Science Communication course is highly effective at improving the confidence of scientists to engage with non-expert audiences and demonstrates the professional benefits of accessible science communication training.

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Abstract 2675

The Dnase1L3 flexible C-terminal domain and altered charges in the actin recognition site result in broad substrate diversity and resistance to actin inhibition

Jennifer Pousont, *The Pingry School*

Sasha Bauhs, Olivia Buvanova, Benjamin Chung, Mia Cuiffo, Jiya Desai, Kayla Kerr, Valentyn Kurylko, Sienna Patel

In the United States alone, 1 in 15 people are affected by autoimmune diseases. These illnesses are caused when body tissue is attacked by its own immune system, which can occur due to the build up of extracellular DNA after cell death. Most extracellular DNA is cleared by nonspecific endonucleases Dnase1L3 and Dnase1. Dnase1L3 activity, when compromised, cannot be rescued by the presence of Dnase1. While the cellular activity of Dnase1 is heavily inhibited by actin, Dnase1L3 is more proficient in degrading extracellular DNA because it resists actin inhibition. Additionally, Dnase1L3 degrades antigenic forms of DNA, including protein-DNA and lipid-DNA complexes, that Dnase1 cannot degrade. Decreased function of Dnase1L3, even with fully functioning Dnase1, can lead to the development of autoimmune diseases. For instance, the R206C mutation reduces activity of Dnase1L3 and is linked to the development of lupus. To examine the structural basis of the enhanced activity of Dnase1L3 in comparison to Dnase1, researchers crystallized the core domain of Dnase1L3 (Dnase1L3 ΔCTD). The last 23 residues, comprising the flexible C-terminus (CTD), were removed to facilitate crystallization. Several key structural differences are responsible for its resistance to actin. In the central hydrophobic region of Dnase1L3, a bulky Phe-131 disrupts actin binding. An out-of-register ∝-helix and the insertion of amino acids in a loop following this helix result in loss of salt bridge formation and electrostatic repulsion of actin, respectively. The CTD of Dnase1L3 enhances degradation of DNA that is complexed with lipids or antibodies. Researchers examined the structure of the CTD using Nuclear Magnetic Resonance spectroscopy and then modeled this domain together with the core domain using Small-Angle X-ray Scattering. The CTD was found to be a flexible, disordered DNA-binding domain that projects outward from the core domain. It was proposed that the CTD enhances Dnase1L3 degradation of diverse forms of DNA because of its structural flexibility. The Pingry School SMART team used the 3-D modeling program, Jmol, to design a model of Dnase1L3 in order to better understand how the unique structure of Dnase1L3 correlates to its function. This model was custom printed by 3D Molecular Designs. Important differences compared to Dnase1 have been highlighted in our model, and allow for a clearer visualization of the structural features of Dnase1L3 that lend it its enhanced DNA degradation functions. A fuller understanding of the unique structure of Dnase1L3, including the CTD and actin recognition site, will allow Dnase1L3 based therapies to be created to treat autoimmune diseases.

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ASBMB

Abstract 2691

Research-centered learning in a workshop style Cell/Molecular Biology CURE

Laura Diaz-Martinez, *Gonzaga University*

Course-based Undergraduate Research Experiences (CUREs) have been associated with gains on student knowledge, confidence and identification as scientists. By embedding hands-on research opportunities in the curriculum, CUREs provide research experiences to more students than traditional research apprenticeships. However, designing and teaching CUREs is challenging due to the time constraints imposed by the traditional lecture-lab course format. Having a single (2–3 h) lab section per week severely limits the type of research that can be done in a CURE. In addition, it forces TAs/Instructors to spend significantly more time outside of class preparing for CURE labs, compared to non-CURE labs. And, in some instances, students enrolled in CURE labs are also expected to spend additional time outside of class in order to complete their experiments, leading to issues of equity and access for students who might have family or work commitments. The Advanced Cell Biology course at Gonzaga University has been redesigned to be taught as a workshop where students analyze the bioactivity of novel compounds in human cancer cells. In this workshop modality, lecture and lab occur in the same physical space: the lab. Lecture time is minimized by using a flipped classroom approach combined with in-class active learning. Students read topics ahead of time, clarify ideas during a mini-lecture and then devote the rest of the time to their research projects while also applying their knowledge through a series of self-paced worksheets. By minimizing lecture time, students have more hands-on time to conduct their research projects, design their own experiments and learn advanced techniques (e.g., cell culture, quantitative confocal microscopy, flow cytometry) that are typically too time consuming for a traditional CURE. In the past two semesters, students in the course have identified ten novel compounds that reduce cell viability in human U2OS (osteosarcoma) cells with IC50s of <10 uM. In addition, students have used their knowledge of molecular/cell biology topics discussed in class to formulate their own hypotheses on potential mechanisms of action for the compounds and design their own experiments to test their hypothesis. Experiments have included testing for potential effects of the compounds on DNA damage, cell cycle progression and cell migration, among other topics. Importantly, the compounds being tested in the Advanced Cell Biology course were synthesized at Gonzaga in the Organic Chemistry labs, which are required for all biology majors. Thus, by linking these courses students are able to take a compound from synthesis to testing, providing a glimpse of the different types of research involved in drug design and development.

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Abstract 2698

Developing a course-based undergraduate research experience (CURE) in protein engineering

John Weldon, *Towson University*

Over the past five years, a grant from Howard Hughes Medical Institute has enabled Towson University to implement a curriculum change that has brought authentic research experiences to a large, diverse group of students. This program, called TU-REP (Research Enhancement Program), is based on studies suggesting that authentic research experiences are better than traditional courses at recruiting and retaining students in STEM fields. As part of TU-REP, faculty participate in professional development focused on inclusive teaching and mentoring while developing a course-based undergraduate research experience (CURE). Over 20 novel CUREs have been employed under TU-REP since its inception in 2017, and many have been offered multiple times during that period. My participation in TU-REP was the development a CURE to replace a traditional biochemistry laboratory course with a research experience that revolved around engineering a protein for improved thermodynamic stability. This CURE used the overall strategy of 1) design, 2) produce, and 3) test mutant proteins predicted to be more thermodynamically stable than the wild-type for use therapeutically. Student groups were presented with the overall task and asked to select and test different point mutations using a web-based stability prediction algorithm. Genes for the best theoretical candidates of each group were created using site-directed mutagenesis. Proteins were recombinantly expressed in *E. coli* with a hexahistidine tag and purified using affinity chromatography on an AKTA FPLC. Thermodynamic stability was evaluated by CD spectroscopy using a concentration gradient of guanidine. An activity assay to ensure that the protein remained functional was planned, but time constraints prevented its execution. Traditional biochemistry laboratory techniques (SDS-PAGE, chromatography, spectroscopy, buffer preparation, pH titration, and others) were covered within the context of the project, allowing students to directly connect these techniques to their practical application. For the current project, we utilized the catalytic domain of *Pseudomonas exotoxin* A, but the strategy could be employed on any protein that is easily manipulated. Course assessments included both individual assignments, such as periodic lab notebook evaluation and quizzes, as well as group assignments, such as lab reports and a final poster presentation to the Towson University community at a student research symposium. Student responses to the course were positive. They appreciated the level of involvement and control over the project, and they were excited to be included in novel scientific research. Future implementation of the CURE will use the lessons learned to improve student engagement and streamline the project workflow.

This work was supported by the Towson University Department of Chemistry, the TU Research Enhancement Program, and the HHMI Inclusive Excellence grant program.

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Abstract 2730

Undergrads Understanding RNR Using 3D Modeling

Jermain Berry, *Grand View University*

Sifa Costansi, Jaren Obia, Jaren Obia, Elizabeth Joe, Ellie Sentselaar, Cole Ward, Laura Rusch-Salazar, Bonnie Hall

Ribonucleotide reductase (RNR) is a key enzyme to prepare for DNA synthesis, catalyzing the production of deoxyribonucleotides from ribonucleotides. As part of an undergraduate course, we chose to focus on the RNR enzyme and study more about how it functions. With our chemistry backgrounds ranging from no college chemistry to Organic Chemistry I, it was a challenge for us to comprehend how an enzyme as complex as RNR worked. Ultimately we chose to use 3D modeling to help us better understand the primary literature we were reading. We learned how ATP binding drives the formation of an α2ß2 complex, which is then capable of catalyzing the necessary radical chemistry. Binding by dATP allosterically inhibits RNR by shifting its form to a stable α6 ring structure that excludes the ß2 complex. Utilizing a cryo-EM structure from the Drennan lab we built two models: one of the active α2ß2 RNR complex and another of the inactive α6 ring form. Throughout this project we explored topics ranging from introductory chemistry through biochemistry. Discussions were driven entirely by our requests to know more about specific concepts. Based on our experiences in this course, we highly recommend using 3D modeling to help students learn and understand topics in chemistry and biochemistry.

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Topic Category Enzyme Chemistry and Catalysis

Abstract 139

Old Yellow Enzymes Reduces Oximes via Imines: A Mechanistic Insights from QM/MM Simulations

Amit Amit, *University of Graz*

Nakia Polidori, Karl Gruber

Biocatalysis is integral to scientific and technological advancement, aiming for sustainable solutions. Ene-reductases catalyze the C = C reduction and are biocatalysis' widely used enzymes. Recently, ene-reductases from the Old Yellow Enzymes (OYE) family were reported catalyzing the reduction of oximes to amines. However, the structural and mechanistic details are yet to be investigated. We are pursuing a hybrid approach combining experimental 3D structure determination with molecular modeling techniques. Together, we are revealing the mechanistic details of oxime reduction by OYE, where the contribution of active site residues (Y27, H178, H181, Y183) has been investigated, and more catalytically efficient variants (Y27F and Y183F) of the enzymes are suggested. Additionally, an intramolecular hydrogen bonding in the substrate facilitates the favorable binding mode that could guide better substrate engineering. Overall our results would advance sustainable and effective solutions for reducing pharmaceutical oxime intermediates.

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Abstract 145

Nitrone Formation by a Flavin-Dependent Monooxygenase

Pablo Sobrado, *Virginia Tech*

Sydney Johnson, Hao Li, Hannah Valentino

OxaD is a flavin dependent monooxygenase (FMO) that converts the indole alkaloid and mycotoxin, roquefortine C, to the nitrone-containing roquefortine L. Previous work has proposed that the reaction of OxaD occurs in two iterative oxidations where a hydroxylamine is the intermediate. Multiple oxidation function in FMOs is very rare and to our knowledge, OxaD is the only known enzyme in its FMO subclass that can carry out multiple oxidations. We investigated the previous proposal of the OxaD reaction scheme as well as the catalytic mechanism in detail. The chemical and kinetic mechanism of OxaD was investigated using rapid-state and steady-state reaction kinetics. Our results showed that OxaD required the presence of the substrate for flavin reduction to occur and a preference for NADPH as the reducing cofactor over NADH. We probed for rate-limiting reaction steps using primary kinetic isotope effect studies. We found that the transfer of the hydride from the NADPH to the flavin cofactor is a rate-limiting reaction step in reduction. The oxidative-half reaction results suggest that the product formation/release and/or flavin dehydration are potential rate limiting steps in the overall cycle. Lastly, we used AlphaFold2 modeling to support our findings and to propose a detailed catalytic mechanism for the unique nitrone-forming monooxygenase, OxaD.

This research was supported by National Science Foundation grant CHE-2003658.

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Abstract 149

Identification of the Critical Role of a Unique Loop in UDGX DNA Glycosylase

Chuan Liang, *Clemson University*

Weiguo Cao, Chenyan Chang

Enzymes from Uracil-DNA Glycosylase (UDG) superfamily are essential to initiate base excision repair to remove DNA base lesions caused by deamination and other reactions. As a new member of the UDG superfamily, UDGX is unique in its ability to crosslink to DNA after uracil excision. This work investigated the functional role of an extended loop in motif 3 of Msm UDGX using mutational, biochemical and kinetic analysis. Besides the previously finding of His109 as a crosslinking point, our study led to several new findings. First, arginine 107 forms an inter-motif salt bridge with aspartate 59 in motif 1. Second, lysine 94 forms a hydrogen bond with asparagine 91 to support its catalytic function. Third, a substitution of glutamic acid for His109 can also crosslink to DNA via forming an ester bond. More importantly, several of mutants (H109A, H109Q and H109E) were found not only affect the crosslinking function, but also the uracil excision function. In summary, the unique loop in motif 3 of UDGX plays multiple and critical roles in uracil excision and post-excision crosslinking. This study reveals an intricate coupling nature of two catalytic functions in UDGX, which offers new insight in understanding multi-function enzymes.

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Abstract 155

Variation in the kinetic parameters and structural features of tumor-driving isocitrate dehydrogenase 1 (IDH1) mutants tune phenotype severity

Christal Sohl, *San Diego State University*

Matthew Mealka, Kaitlyn Sabo, Elene Albekioni, Mowaffaq Adam, Steve Silletti, Mikella Robinson, Carrie House, Tom Huxford

Human isocitrate dehydrogenase 1 (IDH1) regulates cytosolic metabolite levels by catalyzing the reversible NADP+-dependent oxidation of isocitrate to a-ketoglutarate (aKG). Mutations in IDH1 can drive many gliomas, leukemias, and chondrosarcomas typically by ablating the normal reaction and, critically, by catalyzing a neomorphic reaction: the NADPH-dependent reduction of aKG to D-2-hydroxyglutarate (D2HG), an oncometabolite. Most mutations affect residue R132, and mutant IDH1 is a bona fide drug target, with a selective small molecule inhibitor receiving FDA approval. The structural and chemical diversity of IDH1 mutants observed in patients is striking, with mutations including R132H/C/G/L/S/Q. We have recently reported that the steady-state catalytic efficiency of both the normal and neomorphic reactions vary widely depending on the mutant. For example, we show that R132H IDH1, the most common mutation found in patients, has low catalytic efficiency of D2HG production, while R132Q IDH1 maintains some wild type activity and has very robust D2HG production. The structural features that drive these kinetic characteristics are not yet known. Here, we use both static (X-ray crystallography) and dynamic (HDX-MS) structural methods to understand the unique catalytic profiles observed in wild type, R132H, and R132Q IDH1. We highlight important regulatory regions in the three IDH1 forms that have striking differences in local structure and flexibility, and provide evidence of a previously hypothesized but never identified isocitrate pre-active-site binding state in wild type IDH1. Finally, we show that IDH1 mutants with increased catalytic efficiency lead to increased D2HG levels and more severe phenotypes in cell and animal models of IDH1-driven tumors. By establishing the molecular mechanisms of tumor-driving IDH mutations, we can generate tools for predicting patient prognosis and guiding future mutant IDH1 inhibitor design.

This work was funded by a Research Scholar Grant, RSG-19-075-01-TBE, from the American Cancer Society(C.D.S.), National Institutes of Health R35 GM137773 (C.D.S.), the UCSD/SDSU Partnership U54CA132384 (SDSU) & U54CA132379 (UC San Diego), as well as the California Metabolic Research Foundation (SDSU) and the Rees-Stealy Research Foundation (E.A.).

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ASBMB

Abstract 1166

Identification and Comparison of Hydrolases Secreted from *Naganishia albida* to *Papiliotrema laurentii* and their activity toward natural and synthetic polyesters

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Justin Biffinger

Esters and amides are the most versatile functional groups in nature and in plastics today. Understanding the key temporal events in the biodegradation and susceptibility of polymers and plastics (e.g. polyester and polyester polyurethanes) to hydrolytic enzymes is important to the sustainable development of our modern society. We will present the isolation, identification, and classification of an environmental strain of *Naganishia albida* found on polymer coatings inside of aircraft. To better understand the capability of hydrolytic enzymes to degrade plastics we isolated, identified, and classified an environmental strain of *Naganishia albida* found on polymer coatings inside an aircraft. We utilized cultivation conditions that induced the secretion of several hydrolytic proteins (between 30 kDa and 140 kDa) from *N. albida* that were identified via LC-MS. We also isolated the hydrolytic proteins from cellular supernatants using size exclusion chromatography, and performed soluble colorimetric esterase assays and polyester polyurethane coating degradation experiments. Finally we showed comparisons between the hydrolytic proteins produced by *N. albida* and another polymer degrading yeast, *Papiliotrema laurentii*.

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Abstract 1167

Bottom-up Approach to Rebuild Synergy in Biofilms to Stimulate the Degradation of Recalcitrant Polymer Coatings

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Justin Biffinger

The movement toward more environmentally friendly approaches to thwarting biofilm formation on marine and aerospace equipment has led to a more comprehensive approach to deconstructing and identifying the complex interactions with biofilms on polymer coated surfaces. We will present results from the biodegradation of defined thermoplastic polyester polyurethane and polyether polyurethane coatings using single environmental strains of *Bacillus megaterium*, *Papiliotrema laurentii*, and defined mixtures of these two organisms as both liquid cultures and as biofilms on polymer coated surfaces. We will show the changes in population dynamics using a quantitative PCR approach and selective agar plate based assays using antifungal or antimicrobial agents. We will present how under nutrient limited conditions these two organisms appear to control their activity and population density to survive on the carbon sources released from the successful hydrolysis of the synthetic coating. These data show how a prokaryote and eukaryote originally isolated from the same environmental consortium inside of an aircraft interact to affect the degradation of a coated surface over time.

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Abstract 1203

Characterization of a Novel Methanogen Radical SAM enzyme containing a SPASM Domain

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Methanogens depend on their numerous metalloenzymes to catalyze reactions such as the reduction of carbon dioxide to methane, the reduction of dinitrogen to ammonia, and the reversible oxidation of hydrogen to protons. These reactions require metals such as iron, nickel, molybdenum, and cobalt, in addition to sulfur, for completion, yet we have limited knowledge of how these metals, particularly iron and sulfur, are acquired. Recent work has demonstrated that model methanogens *Methanococcus voltae* and *Methanosarcina barkeri* are able to utilize pyrite (FeS2), a mineral thought to be biologically unavailable, as their sole source of iron and sulfur. Existing efforts have focused on understanding how these inorganic nutrients are acquired, trafficked, and utilized by these methanogens. In this study, we have identified upregulated proteins that are potentially involved in these processes from shotgun proteomics experiments and have concentrated on biochemically and biophysically characterizing the products of genes Mvol_0694, Mvol_0695, and Mvol_0696. We have overexpressed these genes in *E. coli*, and through sequence analysis, High-performance Liquid Chromatography (HPLC), and Electron Paramagnetic Resonance (EPR) spectroscopy have determined that the Mvol_0696 gene product is a radical S-deoxyadenoyl-L-methionine (SAM) enzyme with a SPASM domain, meaning that it binds multiple [4Fe-4S] clusters. We hypothesize that this protein utilizes its [4Fe-4S] clusters for the modification of its proposed substrates, the protein products of Mvol_0695 and Mvol_0694. Current work has focused on developing assays utilizing mass spectrometry, HPLC, and EPR to provide experimental evidence to support this hypothesis, including how the Mvol_0694 and Mvol_0695 proteins are modified, and how these modified proteins are involved in the acquisition, trafficking, and storage of [Fe-S] clusters. A thorough understanding of these pathways and modifications will provide insights into early biogeochemical cycles and elucidation of fundamental life processes of these ancient organisms, potentially allowing for sustainable biosynthetic mining of metals in the future.

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Abstract 1245

Enteroviral 2C protein is an RNA-stimulated ATPase and uses a two-step mechanism for binding to RNA and ATP

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The enteroviral 2C protein is a therapeutic target, but the absence of a mechanistic framework for this enzyme limits our understanding of inhibitor mechanisms. Here we use poliovirus 2C and a derivative thereof to elucidate the first biochemical mechanism for this enzyme and confirm the applicability of this mechanism to other members of the enterovirus genus. Our biochemical data are consistent with a dimer forming in solution, binding to RNA, which stimulates ATPase activity by increasing the rate of hydrolysis without impacting affinity for ATP substantially. Both RNA and DNA bind to the same or overlapping site on 2C, driven by the phosphodiester backbone, but only RNA stimulates ATP hydrolysis. We propose that RNA binds to 2C driven by the backbone, with reorientation of the ribose hydroxyls occurring in a second step to form the catalytically competent state. 2C also uses a two-step mechanism for binding to ATP. Initial binding is driven by the α and β phosphates of ATP. In the second step, the adenine base and other substituents of ATP are used to organize the active site for catalysis. These studies provide the first biochemical description of determinants driving specificity and catalytic efficiency of a picornaviral 2C ATPase.

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ASBMB

Abstract 1263

Inhibition of Carbonic Anhydrase Activity by Green Tea antioxidant EGCG

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Previous studies have shown that carbonic anhydrase (CA) and its isozymes contribute to development and progression of cancer. In several studies, an antioxidant from green tea (-)-Epigallocatechin 3-O-Gallate (EGCG) has been shown to slow down proliferation of cancer cells. We hypothesized that this effect of EGCG may be due to inhibition of CA. In addition, we tested if the products of EGCG hydrolysis, Gallic acid, and (-)-Epigallocatechin inhibit Carbonic Anhydrase. Using the electrometric Wilbur-Anderson assay, we successfully measured the CA activity and showed its inhibition by acetazolamide, a known CA inhibitor. The assay involves determination of the time T in seconds required for 4 mL of water saturated with carbon dioxide to lower pH from 8.3 to 6.3 when added to 6 mL of 0.02 M Tris buffer at 0°C without (T0) and with (TE) the enzyme. The activity is defined as A = 2*(T0/TE-1) assay units (AU). In 8 blank trials, T0 was 56 ± 3 s (Mean ± SD), and with 0.001 mg/10 mL (∼3.3 nM) CA, TE = 23 ± 1 s (3084 ± 297 Wilbur-Anderson units). The same concentration of carbonic anhydrase in the presence of 10 μM EGCG resulted in T = 37 ± 1 sec (1095 ± 146 Wilbur-Anderson units), $p < 0.001$. In the presence of 10μM Gallic Acid, T = 18.4 ± 0.4 sec (3970 ± 204 Wilbur-Anderson units), $p = 0.391$. In the presence of 10 μM (-)-Epigallocatechin, T = 20 ± 2 sec (3606 ± 594 Wilbur-Anderson units), $p = 0.320$. These results show that EGCG partially inhibited CA, reducing its activity by 35%. The products of EGCG hydrolysis, Gallic acid, and (-)-Epigallocatechin did not affect carbonic anhydrase activity. This supports our hypothesis that cancer protective properties of EGCG may be in part due to its inhibition of CA. Thus, EGCG and its derivatives could potentially be used for cancer therapy. This research was funded by Wehner Research Fund, MSU College of Science and Mathematics Summer Student Research Program, and the Garden State Louis Stokes Alliance for Minority Participation Program.

This research was funded by Wehner Research Fund, MSU College of Science and Mathematics Summer Student Research Program, and the Garden State Louis Stokes Alliance for Minority Participation Program.

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Abstract 1268

Kinetic and Structural Characterization of Human Isocitrate Dehydrogenase 1

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Isocitrate dehydrogenase 1 (IDH1) is a cytosolic enzyme that helps maintain the redox environment and metabolite pools in cells. At physiological pH, IDH1 forms a catalytically competent homodimer that converts isocitrate (ICT) to α-ketoglutarate (AKG) in an NADP+-dependent oxidative decarboxylation reaction. Mutations in IDH1 typically lead to the neomorphic reduction of AKG to D-2-hydroxyglutarate (D2HG) and are associated with multiple tumor types. Mutations usually affect the active site residue R132, which takes part in coordinating the ICT substrate. However, it is still unclear specifically what kind of conformational changes drive the normal and neomorphic catalytic processes. Here, we use hydrogen/deuterium-exchange mass spectrometry (HDX-MS) to directly analyze structural dynamics and solvent accessibility in IDH1 by allowing active exchange between backbone amide hydrogen atoms and surrounding D2O. Though HDX-MS is limited to peptide resolution, comparing the fluctuations in deuteron uptake by holo and apo wild type IDH1 reports on the local environments of residues involved in intermolecular hydrogen bonding, substrate coordination and catalysis. We hypothesized that local changes in ICT coordination, metal binding, and global changes from the open, inactive to closed, active conformation will depend on which substrate, and perhaps even which order the substrate is added. We show that the most drastic changes in deuteron uptake were observed around the regulatory domain, and, moreover, neither Ca2+ nor ICT alone caused these changes. The highest uptake difference when compared to apo IDH1 was observed when all substrates were bound. NADP+ has been reported to be co-purified with IDH1, and our HDX-MS data supports this, as the deuteron uptake difference between WT IDH1:Apo versus WT IDH1:NADP+ is insignificant. Metal binding data revealed very interesting patterns, such as outer, large domain portions of the enzyme becoming more solvent exposed when Ca2+ binds, while the regulatory domain experienced a decrease in deuteron uptake. Changes in individual substrate and metal binding supported the prediction that ICT binding drives some structural changes that were different from the fully closed, active conformation, and only upon metal and substrate binding was the fully catalytically competent structure achieved. We then extended our interest in observing the mechanism of forward and neomorphic reactions and relative activity of different important mutants. Here, we used spectroscopic tools to measure kinetic properties of IDH1 by monitoring the rate of NADPH formation and consumption in forward and neomorphic reactions respectively. We compared catalytic efficiency of wild type, R132H and R132Q IDH1 mutants, and wild type/

mutant heterodimers, and showed that heterodimerization induced accumulation of greater amount of NADPH compared to the mutant homodimers with the same concentration. Understanding how kinetic rates compare in the IDH1 homodimer and heterodimer complexes and the role in structural dynamics in catalysis as monitored using HDX-MS allow us to better understand the molecular mechanisms of IDH1 catalysis in health and disease.

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Abstract 1279

Utilization of the NAD Cofactor Binding Energy for Stabilization of Transition States for Enzyme-Catalyzed Hydride Transfer Reactions

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The binding energy of the adenosine diphosphate (ADP) fragment of the NAD cofactor is utilized to drive large changes in the conformation of many enzymes that catalyze hydride transfer to NAD. The mechanistic rational for these conformational changes maybe the same as for conformational changes driven by phosphodianion, adenosyl and coenzyme A fragments of substrates for a variety of other enzyme-catalyzed reactions. We report the kinetic parameters for hydride transfer from formate and 3-phosphoglycerate, respectively, to the truncated cofactor nicotinamide ribose (NR) catalyzed by formate dehydrogenase (FDH) and glycerol-3-phosphate dehydrogenase-catalyzed (GPDH); and, kinetic parameters for activation of these hydride-transfer reactions by the cofactor piece adenosine monophosphate (AMP). Removal of the ADP from NAD results in 11.3 and 13.0 kcal/mol increases, respectively, in the activation barrier for hydride transfer catalyzed by FDH and GPDH. The truncated substrate piece adenosine monophosphate (AMP) acts to rescue the lost activity and provides 5.6 and 7.4 kcal/mol stabilization, respectively, of the transition states for FDH- and GPDH-catalyzed hydride transfer to NR at a 1.0 M standard state. These results support the conclusion that FDH and GPDH have evolved to use the large molecular weight cofactor NAD as the common substrate in hydride transfer reactions, because the binding energy for smaller substrate fragments is not sufficient to support rapid turnover of metabolic substrates. We propose that the non-reacting fragment provides a similar stabilization of the transition states for other enzyme-catalyzed hydride transfer reactions by a mechanism where the ADP binding energy is utilized to stabilize the conformation of the catalytically active form of the enzyme.

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Abstract 1285

Probing D2-HG in promoting cancer phenotype severity

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According to the National Cancer Institute, there were 25,050 new brain cancer cases in 2022 in the US, with only 33% percent of patients surviving to 5 years. Human isocitrate dehydrogenase 1 (IDH1) catalyzes the NADP+-dependent conversion of isocitrate to α-ketoglutarate, and mutations in this enzyme have been identified in several tumor types. Mutant IDH1, which catalyzes the NADPH-dependent conversion of α-ketoglutarate to D2-hydroxyglutarate (D2HG), drives >80% of lower-grade gliomas and secondary glioblastomas. IDH1 mutations occur primarily at residue 132, and and we have previously shown how the catalytic activity differs widely among IDH1 mutants. Here, we seek to understand how the unique kinetic profiles among the IDH1 mutants translates into *in vitro* and *in vivo* phenotype severity. Understanding differences among IDH1 mutations will shed light on cancer progression, patients' prognosis, and treatment response. We hypothesized that catalytically efficient IDH1 mutations would have higher cellular levels of D2HG, magnifying tumor phenotype severity. We created stable cell lines expressing IDH1-R132Q and IDH1-R132H mutations in glioma and fibrosarcoma cell lines. Cells expressing IDH1-R132Q produced higher cellular levels of D2HG, leading to lower rates of proliferation and migration. D2HG interferes with DNA and histone demethylation by inhibiting demethylases, and we observed higher levels of methylated histones in IDH1-R132Q compared to IDH1-R132H and IDH1-wild type. We identified elevated D2HG levels in mouse xenografts expressing IDH1-R132Q, leading to higher tumor volume and faster tumor growth. This work helps elucidate the catalytic consequences of IDH1 mutations and resulting cellular concentrations of D2HG., ultimately, and how this might affect patient prognosis.

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Abstract 1305

Elucidating signal transduction pathway in light-activated adenylyl cyclase using unnatural amino acid mutagenesis

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There is extensive research which explores the photochemistry of Blue Light Using FAD photoreceptors, however there is limited literature available on how the signal is transduced from the BLUF domain to the functional/output domain. A detailed understanding of their operation is needed to direct applications such as the development of optogenetic devices. Here, we elucidate the signal transduction pathway of the photoactivated adenylate cyclase OaPAC, in which a BLUF domain is fused to an adenylate cyclase (AC) domain that catalyzes the light-dependent formation of cAMP from ATP. OaPAC has very low dark state activity and is therefore a promising starting point for optogenetic applications. We used unnatural amino acid mutagenesis to explore how light absorption by the N-terminal BLUF domain on the fs timescale leads to activation of the C-terminal adenylate cyclase (AC) domain. We incorporated the IR probe azidophenylalanine (Az-Phe) at F103, W90, F180 and F131 using orthogonal aminoacyl-tRNA-synthetases. The FTIR light minus dark difference spectrum shows that the environment around F103 and W90 residues changes upon light activation, however, there is no change in the environment around F131 upon photoactaction. The enzymatic activity of these mutants was not affected due to the IR probe incorporation. Residue F180 forms π-π stacking interactions with F197 in the AC domain and incorporation of Az-Phe led to complete loss of enzymatic activity upon blue light irradiation. We also replaced Y125 with fluorotyrosine analogues to increase the acidity of the phenolic hydroxyl group and probe the role of Y125 in signal transduction which forms an intersubunit hydrogen bond with N256 from the other monomer in the OaPAC dimer. OaPAC with 3-fluorotyrosine, 3,5-difluorotyrosine or 2,3,5-trifluorotyrosine incorporated at Y125 showed 10 fold higher activity in the dark compared to wild-type OaPAC, whereas Y125F and Y125S showed no dark state activity, implying that the intersubunit hydrogen bond between Y125 and N256 is critical for signal transduction. Finally, we showed that the AC domain become inactive 3 s after irradiation is discontinued, indicating that the photoreceptor responds rapidly to light, thus reinforcing the utility of the protein as a starting point for optogenetic applications.

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Abstract 1343

A Defined Lysate-Free *in vitro* [FeFe]-Hydrogenase Maturation

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Hydrogen (H2) is an energy carrier molecule and a promising alternative to traditional renewable energy technologies when produced in an environmentally-safe system. Hydrogen biosynthesis is achieved by hydrogenases in nature and [FeFe]-hydrogenases are the most efficient class that catalyze hydrogen production from protons and electrons. The active site H-cluster of [FeFe]-hydrogenases (HydA) is composed of a [4Fe-4S] cluster covalently linked to an organometallic dinuclear 2Fe subcluster ([2Fe]H) via a cysteine thiolate ligand. The [4Fe-4S] cluster is assembled by iron-sulfur cluster machinery proteins. However, the assembly and transfer of the [2Fe]H subcluster requires three dedicated maturases: the radical S-adenosyl-L-methionine (SAM) enzymes HydG and HydE, and the GTPase HydF. Previous methods developed for the *in vitro* maturation of HydA emphasized the requirement of *E. coli* lysate in the maturation reaction, presumably to provide components involved in the assembly of the dithiomethylamine (DTMA) bridge of the H-cluster. Fractionation of the lysate followed by shotgun proteomics analysis and hydrogen production assays indicated that aminomethyl-lipoyl-H-protein (Hmet) of the glycine cleavage system (GCS) supports the maturation of HydA in the absence of cell lysate. Further, including in situ Hmet regeneration components, serine hydroxymethyltransferase (SHMT), and the T-protein enabled us to achieve mature HydA with specific activities as high as 390 μmol H2 min-1 mg-1 HydA. Maturation reactions containing isotope-labeled serine (13C3-15N) and ammonia (15NH4) demonstrated that the nitrogen and carbons of the dithiomethylamine (DTMA) moiety are derived from ammonia and serine, respectively. Developing a lysate-free maturation system provides a more definitive understanding of biological H-cluster assembly and better control over future bio-hydrogen production technologies.

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Abstract 1358

The influence of surface residues on the structure and activity of a salt-dependent halophile enzyme

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Halobacterium salinarum (*H. Salinarum*) is a halophilic archaea that grows at high salt concentrations. *H. salinarum* contains an intein that interrupts the DNA PolII protein. Inteins mediate the process of protein splicing, a post-translational modification in which the intein is self-catalytically removed from two flanking extein sequences and the exteins are ligated together. The *in vitro* protein splicing activity of the intein requires high salt to function of at least 1.5 M NaCl. By comparing this intein to those from other non-halophilic extremophiles, we identified nine surface residues that were negatively charged in the *H. salinarum* intein but either positive or neutral in the homologs. We found that a mutant intein with these nine changes can splice at 0.5 M NaCl. We were further able to narrow this activity to two specific residues, suggesting that only two amino acid changes can trigger a substantive folding and activity difference. In addition, we used native Trp fluorescence to study the folding of the inteins as a function of salt concentration; the mutant is well folded at relatively low salt.

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Abstract 1360

CryoEM structures of the nitrogenase complex during catalytic turnover

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The enzyme nitrogenase couples ATP hydrolysis to the multi-electron reduction of atmospheric dinitrogen into ammonia. Despite extensive research, the mechanistic details of ATP-dependent energy transduction and dinitrogen reduction by nitrogenase are not well understood. This is due to the fact that the nitrogenase catalytic activity requires complete turnover conditions, including the presence of two component proteins – Fe-protein (FeP) and MoFe-protein (MoFeP) – and continuous ATP hydrolysis. Substrates can only bind reduced forms of the catalytic center (FeMoco) in MoFeP, however, such reduced forms of FeMoco promptly return to the resting state through H2 evolution upon termination of ATP hydrolysis, rendering substrate/intermediate-bound states of FeMoco too fleeting for crystallographic characterization. Additionally, the necessity of continuous ATP hydrolysis for catalysis leads to a heterogeneous distribution of nitrogenase sub-states which are difficult to interrogate spectroscopically. Furthermore, these spectroscopic investigations only report on the interactions between FeMoco and substrates, yielding little information on how these local interactions may be linked to, and potentially controlled by, global ATP/FeP-dependent structural dynamics of MoFeP. Toward overcoming these challenges, we have interrogated the structural dynamics of the nitrogenase complex by cryogenic electron microscopy (cryoEM) under enzymatic turnover conditions. Our structures show that asymmetry governs all aspects of nitrogenase mechanism including ATP hydrolysis, protein-protein interactions, and catalysis. Furthermore, they reveal several previously unobserved, mechanistically relevant conformational changes near FeMoco that are correlated with the nucleotide-hydrolysis state of the enzyme.

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Abstract 1379

Cloning Disease-Linked Mutations in a Predicted Lipid-Binding Domain of Opa1 Protein

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Autosomal Dominant Optic Atrophy is the most commonly inherited form of optic neuropathy. One of the main causes of this disease are mutations in OPA1, a gene which encodes the mitochondrial-resident enzyme Opa1, a dynamin superfamily protein (DSP). Opa1 is responsible for fusing the inner mitochondrial membrane in mammals. Mutations in Opa1 are believed to be associated with such damaging effects as increased free radicals and decreases in ATP production, thus explaining the role mutations play in loss of vision. One location in the enzyme where disease-causing mutations are especially enriched is a region which we believe is involved in direct interactions with the mitochondrial membrane. This region contains conserved amino acid sequences with a related DSP. To determine the role that some of these disease-causing mutations (namely C841-L887del and R836W) play in disease, we will study them *in vitro* by probing the enzymatic rate, self-assembly, and lipid interactions. We speculate that mutations in the predicted lipid-interacting region of Opa1 will result in no change to self-interaction and limited differences in enzyme rates, but that there will be decreased binding to certain membrane lipids. The current focus on the project is in steps of cloning. While successful cloning of the mutation has occurred, this work is in the process of being repeated due to a non-sense mutation in the template DNA.

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Abstract 1392

Elucidating physiological substrate to probe mechanism of biogenesis of 5-carboxymethylaminomethyl-2-thiouridine in tRNA by MnmEG

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Anthony Young, Vahe Bandarian

The evolutionarily conserved bacterial proteins MnmE and MnmG (and their homologs in eukarya) collectively install a carboxymethylaminomethyl (cmnm) or a taurinomethyl (τm) group at the fifth position of wobble uridines of several tRNA species. The seemingly simple reaction uses two proteins, two substrates, two cofactors and nucleotide hydrolysis to install the cmnm (or τm) group onto tRNA in a complicated manner. The reaction uses a tetrahydrofolate derivative, glycine (or taurine in humans), FAD, NADH and GTP to install the carboxymethylaminomethyl group. Although the MnmEG reaction has been known for over two decades, critical details of the reaction, including the one-carbon donor, and the mechanistic details, have remained elusive. Using isotopically enriched THF substrates and *E. coli* MnmEG model system, we show methyleneTHF as the true substrate for the reaction. Moreover, a reductant is required for the reaction, which can be NADH *in vivo* but can be bypassed by DTT *in vitro*. Using a synthetic flavin enzyme intermediate, we show that FAD acts as the methylene-transferring agent from methyleneTHF to the fifth position of the uracil base. These results collective lead to a mechanistic proposal for the reaction catalyzed by MnmEG and its classification as a FAD/folate-dependent methyltransferases.

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Abstract 1423

Random Expansion of Substrate Specificity of Biocatalytic Esterase

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R. Jeremy Johnson

Esterases are essential enzymes involved in numerous cellular functions such as recycling proteins and mediating signaling pathways. Esterases are also utilized as biocatalysts in the industrial manufacturing of pharmaceuticals and detergents. BS2, an enzyme from *Bacillus subtilis*, is a highly versatile esterase with a distinct ability to hydrolyze esters of tertiary alcohols, which are not hydrolyzed by human esterases. Combining this unusual reactivity for tertiary esters with the ability to split BS2 into reconstituting half sections has made BS2 into a tool for studying protein-protein interactions in human cells. In this study, our goal was to expand the biocatalytic properties and biotechnological applications of BS2 by further expanding its substrate reactivity with diverse ester substrates. Using the three-dimensional structure of BS2 and a comprehensive alanine scan of its binding pocket, we chose multiple hotspots that regulate the substrate specificity of BS2. Utilizing Golden Gate Mutagenesis, randomized combinatorial libraries were synthesized across these binding pocket residues and proper library diversity confirmed by sequence analysis. Random libraries were then screened via pooled high throughput fluorescence assays against a subset of orthogonal ester substrates. Matched BS2 variants and ester substrates will make valuable tools for sensitive visualization of protein-protein interactions and as novel biocatalysts.

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Abstract 1443

Dynamic loop movements in a bacterial APT

William Weiss, *Butler University*

R. Jeremy Johnson

Acyl protein thioesterases (APTs) catalyze the depalmitoylation of proteins inserted into the cell membrane, including critical cancer signaling proteins. To perform this depalmitoylation, APTs must perform two functions concurrently: they have to be bound to the cell membrane and be catalytically active. As a model system for the structure and function of human APTs, in this study we used FTT258, a bacterial APT homologue from *Francisella tularensis*. FTT258 functions as a good model for APT function because of its structural and sequence similarity to human APTs. In this investigation, we sought to directly measure shifts in the protein conformation of FTT258 using intrinsic tryptophan fluorescence in response to biological relevant conditions, including ligand and membrane binding. To isolate individual tryptophans within FTT258, every tryptophan was individually and combinatorially exchanged with phenylalanine and shifts in tryptophan fluorescence measured in response to ligand and membrane binding. To confirm that these tryptophan variants did not significantly shift the folded structure of FTT258, a kinetic assay was conducted with an ester substrate. To investigate shifts in the dynamics of FTT258 in response to ligand binding, changes in the tryptophan fluorescence was measured with increasing concentrations of the human APT selective inhibitors 2-Bromohexadecanoic acid (BHDA), ML-348, and ML-349. Further investigation of the dynamics of FTT258 was performed in response to a membrane-mimicking surfactant, changes in tryptophan fluorescence was measured in response to increasing concentrations of the surfactant Sodium Taurodeoxycholate (NaTDC). These four substrates were used to identify how ligand or membrane binding would affect the movement of a key β3-loop from the inactive to active state. Ongoing investigations will determine if shifts in tryptophan fluorescence are directly connected with β3-loop movement and are connected with the biological function of APTs, all with a broader goal of understanding their role in cancer cell proliferation.

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Abstract 1453

Dynamics loop control the catalytic and ligand binding activity of acyl-protein thioesterases

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William Harris

Acyl protein thioesterases catalyze the depalmitoylation of key signaling proteins attached to the plasma membrane. Human APTs use a two-step catalytic mechanism involving initial membrane binding followed by substrate binding and cleavage. This two-step mechanism is regulated by the dynamic movement of multiple flexible loops whose rearrangement dually controls membrane binding and catalytic activity. Herein, we investigated the importance of these loop dynamics to the catalytic activity, substrate specificity, and ligand binding of human APT1. Using alanine scanning libraries across these flexible loops, we measured shifts in the catalytic activity across various ester substrates and confirmed the importance of these residues to direct ligand interaction. APT1 showed highest catalytic toward shorter ester substrates but retained significant activity across a range of substrates up to 16-carbons. Multiple hydrophobic residues positioned in the center of these dynamic loops were most essential to the catalytic activity of APT1 as their removal by even conservation substitutions led to near complete loss of catalytic activity. These hydrophobic residues also interact directly with the ester substrates as substitution of these hydrophobic residues also reduced the thermal stabilization of APT1 upon ligand binding and reduced the inherent IC50 value of APT1 for selective inhibitors. We are now working to correlate these shifts in catalytic activity and ligand binding with concurrent shifts in membrane binding.

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Abstract 1511

The biochemistry of iron-sulfur cluster maturation in *Staphylococcus aureus*

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Jesse Hudspeth, Emily Sabo, Veronica Stark, Amy Boncella

A core mission in the Morrison lab is to create new antibacterial agents that inhibit a novel pathway target in *Staphylococcus aureus*. The pathway under investigation is the SUF-like pathway of iron-sulfur cluster biosynthesis in Gram-positive organisms. The SUF-like pathway and its component proteins are essential in *S. aureus* and do not share homology with analogous pathways in humans. This make the SUF-like pathway an attractive target for the development of new antibacterial agents, which may be broadly applied to other Gram-positive pathogens that exclusively rely on SUF-like pathway, including *Enterococcus faecalis*, *Streptococcus pneumoniae*, and *Mycobacterium tuberculosis*, as well as some parasites that cause malaria. As an initial step toward their goal of creating first-in-class drug candidates targeting the SUF-like pathway, the Morrison lab is characterizing the essential cysteine desulfurase and sulfurtransferase in this pathway in *S. aureus*. Biochemical and structural investigations by the Morrison lab confirm the identity and likely roles of these proteins; however, unexpected activity levels suggest there may be unique features of this pathway in *S. aureus* compared to the well-studied homologous pathway in *Bacillus subtilis*. In this talk, Dr. Morrison will share her lab's work on the biochemical characterization of proteins in the SUF-like pathway in *S. aureus* and outline her approach and progress to design inhibitors of this pathway.

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Abstract 1541

Investigating the substrate-dependent effect of different regulator molecules on SIRT1 activity

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Praagna Doddaballapur, Ningkun Wang

SIRT1, a lysine deacetylase, is a protein from a group of Sirtuins that are found in the human body involved in numerous cellular functions such as aging, neurodegenerative resistance, and insulin secretion. It is known that SIRT1 can be regulated by resveratrol, where the effect of resveratrol on SIRT1 is dependent on the different peptide substrates. The goal of our project is to test if this type of substrate-dependent regulation of SIRT1 would apply to other known sirtuin-activating compounds (STAC). The STACs we are studying specifically are piceatannol and SRT1720 because these two STACs have been shown to increase SIRT1 activity by more than 8-fold at 100 μM and lower concentration. But they have only been tested against a limited number of peptide substrates. Additionally, piceatannol's molecular structure is similar to resveratrol's molecular structure, differing in only an extra hydroxyl group, while SRT1720's molecular structure is significantly different. We use plate reader based enzyme-coupled assays to determine the Michaelis-Menten enzyme kinetics of SIRT1 with the addition of either piceatannol or SRT1720 with various peptide substrates in our library to determine the extent of substrate-dependent regulation. The importance of investigating the substrate-dependent regulation of SIRT1 is to lead to further understanding of how the structure of STACs relate to this substrate-dependent regulation. This can lead to the development of new therapeutics, which can be optimized specifically to target SIRT1 with varying substrates.

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ASBMB

Abstract 1563

Utilizing Isothermal Titration Calorimetry to Measure β-galactosidase Activity in High Lactose Dairy products

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Jason Kenealey

Spectrophotometry has dominated enzyme kinetic assays for the better part of the last century. However, a key limitation of spectrophotometry is that the solution used must be transparent and have limited spectral absorbance. Isothermal Titration Calorimetry (ITC) offers a robust method for measuring enzyme activity in opaque, complex solutions such as dairy products and other food and biological matrices. Rather than measuring product formed as is typical for spectrophotometric assays, the ITC can directly measure reaction progress and can obtain thermodynamic values such as enthalpy. In this study, we used the ITC to observe the heat rate (uW) for β-galactosidase activity on lactose-added substrate such as sterile milk, sweet and acid whey, and whey permeates. Purified enzyme was injected at concentrations of 0.0, 0.44, 0.89, and 1.33 mg/mL to dairy fluids spiked with 500 mM lactose, and the heat rate of the resulting reaction was observed. When compared to the same reaction carried out in sodium acetate or PBS buffer (typical for enzyme kinetics studies) we demonstrated changes in activity resulting from differences in dairy fluid composition, pH, and temperature. For example, β-galactosidases from both *Aspergillus oryzae* and *Kluyveromyces lactis* displayed higher activity in the presence of sweet whey compared to sweet whey permeate, inferring that the presence of proteins may help stabilize or otherwise improve enzyme activity. β-galactosidase from *A. oryzae* was more active at high temperature (60°C compared to 40°C) whereas *K. lactis* had no apparent reaction (consistent with denaturation) at 60°C. Similarly, *A. oryzae* was functional in acid whey whereas *K. lactis* was not. Furthermore, we observed differences in thermodynamic behavior for β-galactosidases from *A. oryzae* and *K. lactis*, which can be attributed to a preference for lactose hydrolysis and galacto-oligosaccharide formation respectively, although HPLC product analysis will be necessary to support this claim. We demonstrated that the ITC is an effective tool for directly measuring enzyme activity in dairy fluids, gives deeper insight into the mechanistic behavior of enzymatic reactions, and offers a new technique for enzyme kinetic studies in complex, opaque solutions such as milk and whey.

We are grateful for BUILD Dairy for funding this research.

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Abstract 1566

Role of Histidine290 in tuning the redox activity of Electron Transfer-FAD in Electron Transfer Flavoprotein of *Acidaminococcus fermentans*

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Anne-Frances Miller

Flavin based electron bifurcation (FBEB), a third mode of energy conservation in anaerobes, produces low-potential energy-rich reducing agents able to drive highly demanding reactions. Electron bifurcation couples an endergonic electron transfer to a more exergonic one, making the overall reaction exergonic. The ETF from *Acidaminococcus fermentans* (Afe) is a heterodimer where subunit EtfA contributes domain I and domain II, and EtfB contributes domain III. The ETF has two FADs: the electron transfer FAD (ET-FAD) and the bifurcating FAD (Bf-FAD). In the elucidated mechanism of bifurcation, NADH donates a pair of electrons to the Bf-FAD which bifurcates one electron exergonically to a high potential acceptor via the ET-FAD and the other electron endergonically to a low potential acceptor. A domain-scale conformational change has been proposed to gate electron transfer and ensure that only one electron per pair accesses the exergonic path. However, if the ET-FAD's resting state is a semiquinone state, then it only has capacity for one more electron, so the second electron of each pair must use the endergonic pathway. Thus, the stability of ET-flavin's semiquinone is critical to the efficiency of bifurcation. The ET-flavin adopts an anionic semiquinone (ASQ) state, although ASQ only accumulates to 1% of the population in free FAD. Thus, the protein environment plays a crucial role in stabilizing the ASQ in ETF. We are studying the interactions of ET-FAD with nearby amino acid residues which could stabilize ASQ. His-290 is appropriately positioned to function as a hydrogen bond (H-bd) donor to the O2 of the ET-FAD, thereby stabilizing excess negative charge in the N1C2 = O2 locus. Our study probes the significance of His-290 in stabilizing ET-FAD ASQ in EtfAB. Additionally, we are intrigued by reports that the ET-FAD of ETF is unusually prone to chemical modification. Since the proposed mechanisms of modification invoked nucleophilic attack on C8M, we postulated the presence of interactions that would heighten the electrophilicity of C8M in the ET-FAD. We hypothesize that replacing the H290 residue in AfeETF would alter the stability of ET-FAD's ASQ in tandem with its susceptibility to modification. Variant AfeETFs where H290 is replaced by residues A, N, E K, T or F, and a double mutant were designed. H290A, E, N, K, T had diminished stoichiometries of FAD, as isolated. The FAD-to-protein ratios ranging from 1 to 2 are consistent with retention of the Bf-FAD and selective weakening of ET-FAD binding. However, our doubly substituted variant accommodates 2 FAD per heterodimer, and has an optical signature resembling the WT. The superior stability of this variant permitted reductive titrations. WT AfeETF displayed three reductive phases during stepwise

reduction with either NADH or dithionite, revealing a lower two-electron (2e) reduction potential (E°) for the Bf-FAD and two higher 1e E°s for the ET-FAD. However, in the doubly substituted variant, reductive titrations with NADH and dithionite yielded considerably lower ASQ accumulation for ET-FAD. Reductive titrations with dithionite also revealed ASQ formation in Bf-FAD based on absorbance spectra. This is noteworthy, as destabilization of the ASQ state of Bf-FAD is deemed crucial for bifurcation. E°s of ET-FAD and Bf-FAD are being determined to learn how H290 shapes the energy landscape of bifurcation in AfeETF. The measurements reveal perturbation of Eo'Ox/SQ and Eo'SQ/HQ of ET-FAD in the variants, suggesting that H290 plays a very significant role in stabilizing the ASQ state of ET-FAD.

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Abstract 1579

A common strategy for bacterial cell envelope polymer acylation involves a multi-enzyme cascade of covalent intermediates

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Bailey Schultz, Eric Snow, Suzanne Walker

Bacterial cells coat themselves with a diverse array of polymers that allow for survival in both hostile and benign environments. Such polymers—typically composed of sugars or sugar alcohols—are often modified post-synthesis through O-acylation, resulting in ester groups that alter the polymers' properties and contribute substantially to virulence and antibiotic resistance in many pathogens. Despite the importance of this class of modification, how these ester groups are installed onto extracytoplasmic polymers using acyl donors generated in the cytoplasm is, for the most part, unknown. Here, we uncover a common mechanism for bacterial cell envelope polymer O-acylation, focusing on D-alanylation of the Gram-positive bacterial polymer lipoteichoic acid as a model pathway. This specific pathway's mechanism, we propose, involves the membrane-bound O-acyltransferase (MBOAT)-mediated transfer of a D-alanyl group from a cytoplasmic thioester to a highly conserved six-amino acid motif that we identified at the extracellular C-terminus of a previously uncharacterized single-pass transmembrane microprotein. The hexapeptide motif then carries the D-alanyl group to the active site of a hydrolase/esterase-like protein for transfer to lipoteichoic acid. Through co-immunoprecipitation, we found that the microprotein acts as a linchpin in a three-protein complex comprising itself, the MBOAT, and the hydrolase/esterase-like protein. Genetic assays then revealed that the microprotein's C-terminal hexapeptide motif is required for lipoteichoic acid D-alanylation in *Staphylococcus aureus*, and *in vitro* reconstitution supported the existence of covalent intermediates on both the microprotein and the hydrolase/esterase-like enzyme. Many acylation pathways across both Gram-positive and Gram-negative bacteria, as well as some proposed acylation pathways in archaea, contain cognate pairs of MBOATs and hydrolase/esterase-like enzymes. While most of these pathways do not contain a microprotein like that found in lipoteichoic acid D-alanylation, we discovered that the conserved C-terminal six-amino acid motif from that microprotein is present at the C-termini of these other pathways' MBOATs. Thus, we propose that the strategy used in lipoteichoic acid D-alanylation is used in other polymer O-acylation pathways across prokaryotes, and we note that the hexapeptide motif central to this strategy could serve as a fruitful starting point for the development of peptidomimetic inhibitors targeting these pathways in pathogens.

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Abstract 1594

A Robust Na+, K+-ATPase Activity Assay for the Evaluation of Synthetic Inhibitors: A Screen for Potential Migraine Mitigation Pharmaceuticals

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Emma Barnes, Min Chae, Morgan Hatlovic, Nhat Huynh, Alexa Morrow, Gregory Smith, Marko Velickovic, Roger Biringer

The focus of the work presented here involves the design and development of a simple, robust NKA assay that can be readily applied to examine large numbers of synthetic ouabain-like compounds. The catalytic activity of purified porcine brain NKA as measured by its ability to hydrolyze adenosine triphosphate (ATP) was evaluated using several different protocols. Modified methods by Sweadner and Taussky-Shore as well as a commercially available Malachite Green assay (Sigma Aldrich) were examined for their ability to reproducibly measure phosphate production by NKA. The efficacy of known NKA inhibitors (ouabain, digoxin, bufalin, ursolic acid, and oleanolic acid) were evaluated over a range of concentrations utilizing a modified Taussky-Shore method and IC50 values obtained. The Sweadner method and Malachite Green assays were found to be non-reproducible and incompatible with the required concentrations of NKA and ATP. A modified Taussky-Shore method was found to be quite robust and inhibition assays using this method produced IC50 values within the ranges reported in the literature. The modified Taussky-Shore method is robust and can readily be used to evaluate potential migraine pharmaceuticals, and the assay is linear through 2000 nmole of phosphate. IC50 values obtained are quite comparable to those obtained from the literature, thus confirming the validity of the assay results.

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Abstract 1603

Mutagenesis of a phycoerythrobilin lyase MpeW to gain isomerase function in Synechococcus sp. A15-62

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Irin Tom, Theophile Grebert, Frederic Partensky, Xiaojing Yang, Wendy Schluchter

The phycobilisomes of marine cyanobacteria contains phycobiliproteins that are ideally suited to absorb light in the blue-green region of the spectrum. Phycobiliproteins, such as phycoerythrin II, are composed α- and β-subunits that have either the green-light absorbing phycoerythrobilin or the blue-light absorbing phycourobilin bound at specific positions. In response to changes in light color, the species Synechococcus A15-62 undergoes a process called Type IV chromatic acclimation. Acquisition of a genomic island permits this organism to alter its bilin content. The lyase-isomerase MpeQ, encoded in the genome, attaches phycoerythrobilin to Cys-83 on the alpha subunit of phycoerythrin II and isomerizes it to phycourobilin in blue light conditions. In green light, the MpeW protein, which is encoded on the genomic island, is upregulated and outcompetes MpeQ in order to attach phycoerythrobilin to that same residue. Structural analysis of MpeQ and sequence alignments of the closely related MpeQWYZ family of lyases and lyase-isomerases suggest that amino acids at positions 100, 224, 319, 320, 323, and 352 confer isomerase activity. These sites in MpeW were mutated to be like MpeQ at the same positions. Wild type and mutant MpeW proteins were co-expressed with the alpha subunit and the enzymes necessary for phycoerythrin synthesis in *E. coli*. The purified samples were analyzed by absorbance and fluorescence spectroscopy. Results suggest that Val319 and Thr 320 are critical for isomerase activity, with increasing isomerase activity with additional mutations at positions 100, 224, 323, and 352 in MpeW.

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Abstract 1608

Coordination chemistry control of coenzyme B12 synthesis

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Zhu Li, Ruma Banerjee

5′-deoxyadenosylcobalamin (AdoCbl or coenzyme B12) serves as a cofactor for methylmalonyl-CoA mutase, which that plays an important role in cholesterol, odd-chain fatty acid and branched-chain amino acid metabolism catabolism. AdoCbl synthesis is catalyzed by ATP:cob(I)alamin adenosyltransferase (ATR), which uses ATP and cob(II)alamin as substrates. ATR binds cob(II)alamin in an unusual 4-coordinate (4-c) state, which is predicted based on computational studies to increase the Co(II)/Co(I) reduction potential by >250 mV. The redox potential for 5-c cob(II)alamin (>510 mV) is challenging for biological reductants. Experimental determination of the 4-c cob(II)alamin/cob(I)alamin reduction potential is challenging as it is formed only in presence of co-substrate ATP and cob(I)alamin reacts rapidly to form AdoCbl. Using a patient R190H variant of ATR, which exhibits an ∼110-fold lower activity, we were able to stabilize the cob(I)alamin intermediate following cob(II)alamin reduction. Spectrochemical redox titration using benzyl viologen yielded the first-ever experimental determination of a 4c cob(II)alamin/cob(I)alamin reduction potential of −325 ± 9 mV. The absence of AdoCbl synthesis during the reaction was confirmed by HPLC analysis. Furthermore, we demonstrated that NADPH-dependent adrenodoxin reductase (AdxR)/adrenodoxin(Adx) can serve as the electron donor to ATR () versus min-1 with titanium citrate in the standard *in vitro* assay. Michaelis-Menten analysis of the ATR reaction yielded a KM of for adrenodoxin. The R186Q and E193K patient mutations of ATR impair NADPH-dependent AdoCbl synthesis reaction. Both R186Q and E193K ATR bind cob(II)alamin in a 5-c state, presumably lowering the redox potential for the cob(II)alamin/cob(I)alamin couple. This study reveals the importance of coordination geometry control by ATR for bringing the reaction catalyzed within the range of biological reductants.

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Abstract 1616

Investigating the Pathways of SIRT1 Allosteric Regulation

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My Vu, Zain Alam, Ningkun Wang

SIRT1 is a lysine deacetylase enzyme that participates in several physiological processes, including insulin release and neurodegeneration, making it a potential therapeutic target for chronic illnesses such as Type II diabetes and Alzheimer's disease. Sirtuin Activating Compounds (STACs), such as resveratrol, can regulate SIRT1 activity allosterically by binding to the N-terminal STAC Binding Domain (SBD). Previous research has shown that resveratrol affects SIRT1 activity differently depending on the peptide substrates, but details for the mechanism of this regulation are lacking. The goal of our research is to evaluate the precise enzyme kinetics of SIRT1 with and without resveratrol against various peptide substrates using the continuous enzyme-coupled assay method. Binding assays and small angle X-ray scattering (SAXS) methods are also utilized to investigate conformational changes between SIRT1 and resveratrol when SIRT1 is in complex with various different substrates. We hypothesize that resveratrol changes SIRT1 activity allosterically by modifying the conformation of the SBD region close to the catalytic core, resulting in a substantial shift in the substrate recognition (KM). When resveratrol is added, a significant change in the KM of SIRT1 with a particular peptide would suggest that resveratrol affected the enzyme's ability to recognize its substrate, whereas a significant change in the kcat may suggest that resveratrol altered the enzyme's ability to catalyze the deacetylation of that particular peptide. The kinetic parameters (kcat and KM) of SIRT1 with these peptide substrates with and without resveratrol were studied and we found that KM contributes more to the activity change. We also measured binding affinity (KD) values between SIRT1 and resveratrol when SIRT1 is complexed with different substrates, and observed that the affinity between resveratrol and SIRT1 is changed significantly when in the presence of different peptide substrates. Both these observations suggest a significant change in SIRT1 conformation upon the addition of resveratrol that is substrate-dependent. SAXS data that directly reflect the conformation of SIRT1 also support this finding. We expect to identify a connection between SIRT1 enzyme activity and the conformational changes of the N-terminal domain by integrating data from Michaelis-Menten parameters, binding assays, and SAXS. Discovering more about SIRT1's allosteric mechanism might help in the development of a drug target for regulating SIRT1 activity in cells.

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Abstract 1630

Cryo-EM structure of the *E. coli* acetyl-CoA carboxylase complex, forming an unprecedented tube-like filament

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The first step of fatty acid biosynthesis is the carboxylation of acetyl-CoA to malonyl-CoA performed by an acetyl-CoA carboxylase (ACC) enzyme complex. Since this reaction is powered by ATP it is highly regulated, yet how the various regulators work is unknown. In addition, the organization of many bacterial and plant enzymes remains unknown. We have determined the Cryo-EM structure of the *E. coli* ACC, which forms unprecedented tube-like complexes with a diameter of ~300 nm. The organization of the biotin carboxylase (BC) domain and carboxyltransferase (CT) domain leads to active sites that are packed near eachother allowing for regulatory interactions. Furthermore, the structure points all of the active sites toward the interior of the filament. While crystal structures of the BC and CT domains were known, the N- and C- termini were disordered. These disordered regions in the crystal structures make contacts that are responsible for holding together the complex and are somewhat variable between bacterial strains. Our structure provide information necessary to dissect various modes of allosteric regulation to support biofuel biosynthesis and provide novel sites to inhibit the ACC of specific bacteria.

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Abstract 1635

Inspiring new NRPS cyclization domain mechanisms: the role of protein dynamics in substrate recognition and allostery

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Nonribosomal peptide synthetases (NRPSs) produce some of nature's most diverse peptides including antibiotics and virulence factors and are thus integral targets for new therapeutics. Their modular, multi-domain architecture allows for bioengineering approaches to produce new peptides through domain swaps and mutagenesis. NRPSs are known to exhibit large-scale domain rearrangements wherein characteristic domains within modules must transiently engage to modify and condense substrates and subsequently pass off the intermediate to the next module. Core to this machinery, two substrates, each attached through a 20-Å prosthetic to a carrier protein (CP) domain, are condensed by condensation (C) or cyclization (Cy) domains, with the latter additionally catalyzing heterocyclization of the intermediate. How C and Cy domains recognize CP domains and their substrates amidst vast interdomain dynamics to form stable complexes remains unknown. To engineer exogenous substrate recognition into C and Cy domains, the molecular mechanisms of native domain and substrate recognition need to be better understood. Using NMR relaxation dispersion, we discovered pervasive dynamics within the cyclization domain Cy1 from yersiniabactin synthetase. Inspection of other C and Cy domain structures highlights a motion of the N-terminal subdomain captured in Cy1 dynamics. To determine the function of Cy1 dynamics, we used NMR to determine its molecular response as a substrate is attached to its partner CP domain in situ. We observed an allosteric response only when the substrate is present. This global response reaches from one CP binding site to the other and encompasses regions critical for catalysis. To demonstrate that dynamics were necessary for this allosteric response, we introduced an activity-impairing mutation and observed a dramatic reduction in domain dynamics and an impeded substrate-specific allosteric response. We conclude that Cy dynamics are critical for giving access to conformations involved in CP domain and substrate recognition. These results highlight the importance of dynamics in allosteric responses and molecular communication and stress the need to account for dynamics when applying mutagenesis.

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Abstract 1662

Investigating E219 mutations in Indole-3-Glycerol-Phosphate Synthase (IGPS) enzyme from *Mycobacterium tuberculosis*

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Nina Goodey, David Konas, Sarah Cho

Tuberculosis is up to this day one of the world's deadliest infectious diseases affecting people worldwide. Each day, over 4,100 people lose their lives to tuberculosis and close to 28,000 people fall ill. More critical, medicine has lost their effectiveness over time, as the bacterium is finding ways to resist the action of drugs. Recent research has provided results that suggest Indole-3-Glycerol-Phosphate Synthase (IGPS) from *Mycobacterium tuberculosis* could be a drug target. Indole-3-Glycerol-Phosphate Synthase (IGPS) is an enzyme involved in the tryptophan biosynthetic pathway. IGPS catalyzes the fourth step in the tryptophan biosynthesis pathway, a process deemed essential for optimal *Mycobacterium tuberculosis* growth and inhibition of this pathway could help effectively fight *M. tuberculosis*. Our research focuses on IGPS from *Mycobacterium tuberculosis* (Mt.IGPS). Through mutagenesis, we specifically study E219D, E219N and E219Q. Comparing steady state kinetic parameters and rate pH profile performed on wildtype E219 and its mutants will provide understanding of the conserved residue E219 role in substrate binding and catalysis. From our results these small modifications impacted the overall Mt.IGPS by significantly reducing its catalytic activity. E219D shows an 81-fold reduction in activity as compared to wildtype. Furthermore, an interesting result is the high Km in the mutants with an amide side chain such as E219N and E219Q when compared to wildtype. This high km uncovers how the substrate does not bind well, as compared to the carboxylic acid side chain mutants. We suggest that perhaps the negative charge "carboxylic group" at E219 is important for substrate binding and this information could be helpful for designing inhibitors. Information of E219 mutations is rather new and not enough previous research exists. That is why further research is important. We truly consider this research and future findings helpful to accelerate innovative medicine, that one day pharmaceutical companies can produce new tuberculosis treatment, specially to target multidrug resistant tuberculosis.

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Abstract 1783

Probing protein arginine methyltransferase (PRMT) activity in mammalian cell lysates

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Ashley Holtz, Steve Clarke

The activities of the family of nine mammalian protein arginine methyltransferases (PRMTs 1–9) are often overexpressed in cancer but it has been challenging to determine the *in vivo* substrates of each of these enzymes, especially with their potential overlapping substrate specificity. Remarkably, it has not been possible to date to readily assay the activity of the individual PRMTs in cell extracts. However, the development of inhibitors for PRMTs with specificity for the individual enzymes has now made possible new approaches. In this study we demonstrate the capture of specific types of PRMT activities in mammalian cell lysates utilizing a P81 phosphocellulose assay and a radioactive gel assay based on newly developed PRMT inhibitors. To parse out potentially unique activities of PRMTs, we have characterized recombinant human PRMT1, PRMT5, and PRMT7 activities *in vitro*. PRMT1 represents the major type I PRMT activity responsible for forming asymmetric dimethylarginine residues, PRMT5 represents the major type II activity forming symmetric dimethylarginine residues, and PRMT7 is the only type III activity limited to forming monomethylarginine residues. We have identified potential regulation of these enzymes by temperature, pH, and ionic strength. PRMT1 has optimal activity at a temperature range of 20 °C–37 °C, a pH of 7.4, and an ionic strength of 0 M. PRMT5 has optimal activity at a temperature of 37 °C, pH at a wide range of 6.6–8.5, and an ionic strength of 0 M. PRMT7 has an optimal activity at a temperature of 15 °C, a pH of 8.5, and an ionic strength of 0 M. Using these conditions with the type I specific inhibitor MS023 at 2 μM, the PRMT5 inhibitor EPZ015666 at 20 μM, and the PRMT7 inhibitor SGC8158 at 0.2 μM, we have measured PRMT activities in mammalian cell lysates. Altogether, our results suggest that PRMT1 is the main enzyme responsible for arginine methylation which supports previous results using knockout cells. However, the activity of PRMT5 in mammalian lysates is quite low in comparison, while PRMT7 activity is difficult to detect. The ability to demonstrate that individual PRMT activities in mammalian cell lysates may be useful to discern the relevance of PRMTs in normal cells and in cancer cells.

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Abstract 1856

Investigating the role of the N-terminal cysteine-rich domain of violaxanthin de-epoxidase like –2 (VDL-2) in redox sensing in diatoms

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Carotenoid pigments in photosynthetic organisms serve both light harvesting and energy dissipating roles. One key enzyme involved in regulating the response of photosynthetic organisms to high light intensity is violaxanthin de-epoxidase, which converts the light harvesting carotenoids violaxanthin to zeaxanthin under high light conditions. Compared to land plants, diatoms contain a wide range of additional carotenoids, such as fucoxanthin and diadinoxanthin, which require an additional suite of enzymes to biosynthesize and regulate. The diatom enzyme violaxanthin de-epoxidase like-2 (VDL-2) has previously been reported to be involved in the biosynthesis of fucoxanthin, the key light harvesting carotenoid in diatoms. VDL-2 is closely related to land plant violaxanthin de-epoxidase enzymes, which share an N-terminal cysteine-rich domain involved in regulation. In this study, we investigate the activity of full-length recombinant VDL-2 from the diatom *Thalassiosira pseudonana* to identify the role of disulfide bonds in the N-terminal domain in regulating enzymatic activity. We successfully isolated full-length VDL-2 enzyme through Ni-NTA and S-200 size exclusion column chromatography. Surprisingly, the purified enzyme has a distinct yellow color from an unknown chromophore absorbing at approximately 406 nm. We utilized 5,5′-dithio-bis-(2-nitrobenzoic acid) to identify the oxidation state of the cysteine residues in the N-terminal domain, and the impact of changes in solution redox poise on the number of free thiols. In the future, we plan to correlate redox state and structure of the N-terminal cysteine-rich domain to the rate of carotenoid turnover of the enzyme.

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Abstract 1858

Active and allosteric site evolution directs ordered water use in kinesin catalysis

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ATP hydrolysis is the requisite chemical entry into mechanotransduction. Tts biochemical propagation from the orthosteric site to other parts of enzyme likely diverges between sequence-related isozymes from different species. Unknown is whether shifts in amino acid occurrence in mitotic kinesins are correlated with adaptation of cell division in cold-blood and warm-blooded organisms. A novel 1.74 Å co-crystal structure of Drosophila kinesin-5 and AMPPNP exhibits a complex water network comprised of conserved and unique molecules resident in the active site, when compared to human kinesin-5. Kinetic isotope experiments (KIE) demonstrate that both kinesin-5 motors utilize proton tunneling involving water molecules during catalysis. However, the pH-dependence of the KIE differed between the Drosophila and human enzymes, which may be linked with unique amino acid utilization between the two isozymes. Furthermore, the Arrhenius temperature dependence of KIE of the Drosophila isozyme exhibited broader temperature tolerance, in comparison to the human isozyme. We conclude that protonation events in kinesin rate enhancement can be thermoadapted via amino acid substitutions and associated changes in active site solvation. The protein composition of the active site recruit water molecules as critical chemical reagents for nucleophilic attack and as vehicles for allosteric communication, such as mechanotransduction. Such molecular control of proton tunneling via water networks may be an adaptive mechanism extendable to other motor ATPases.

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Abstract 1874

Human DNA ligase I active site mutants demonstrate failure in non-canonical ligation

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DNA ligases, responsible for finalizing DNA repair, replication, and recombination, catalyze the final phosphodiester linkage between two adjacent ends of nick DNA in a process referred to as DNA ligation. Although the chemical mechanism of DNA ligation is universally conserved among all mammalian ligases, how a human ligase discriminates against unusual 5′ or 3′ DNA ends to ensure faithful ligation is still not fully understood. Since ligation is the last step in most DNA transactions, thorough comprehension of the molecular mechanisms of DNA ligation is critical to elucidate how genome stability is preserved by DNA ligases. Here we dissect the mismatch discrimination mechanism of human DNA ligase I (LIG1) through biochemical and structural characterization. Using active site mutants of LIG1 carrying A or L mutations at conserved residues F635 or F872, we showed that these ligase mutants are not capable of ligating substrates with all 12 possible mismatches at the 3′-end of nick DNA while still maintaining efficiency against canonical substrates. Structurally, we demonstrated that this lack of catalysis in the F635A mutant is due to a shift in a flexible loop which positions R738 within hydrogen bonding distance of the 5′-end of the nick, causing an increased barrier to adenylate transfer. Our findings contribute towards a better understanding of how LIG1 discriminates against mutagenic repair intermediates at atomic resolution. Overall, our work provides insight into the mechanism that could lead to accurate versus mutagenic ligation in the presence of non-canonical DNA ends during the final step of DNA replication and repair, which is critical for the development of potential therapeutic targets and strategies against human DNA ligases.

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Abstract 1892

Characterization of a 7-deazaguanine-based bacterial modification system

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Restriction-modification (R-M) systems are common strategies bacteria use as anti-pathogen defense against bacteriophages. Typically, these systems contain a methyltransferase (MTase) that modifies a particular sequence of the host DNA and a restriction endonuclease (REase) that cleaves pathogen DNA by identifying the unmodified recognition sequence. Recently we discovered a novel R-M system that introduces two 7-deazaguanine based modifications into the DNA, 2′-deoxy-7-cyano-7-deazaguanine (dPreQ0) and 2′-deoxy-7-amido-7-deazaguanine (dADG). 7-deazaguanine R-M systems are found across a diverse set of bacteria. The *Salmonella montevideo* R-M system comprises eleven different proteins exemplifying the complexity of 7-deazaguanine R-M systems. Three of these, DpdA, DpdB, and DpdC are responsible for the formation of structural modification to DNA, whereas the rest of the proteins are involved in restriction endonuclease activity. We present here the elucidation and preliminary characterization of the proteins involved in the modification component of this R-M system, those responsible for the formation of dPreQ0 and dADG in DNA, and identify a target sequence for the bacterial modification apparatus.

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Abstract 1897

Examining combinatorial biosynthesis with diterpene cyclases and synthases

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Diterpene compounds are present throughout many domains of biology where they serve diverse physiological roles. In addition, a number of diterpene compounds have been co-opted for industrial or pharmaceutical uses (a few examples being stevia as a sweetener, taxol as an anticancer drug, and pleuromutilin as an antibiotic.) To increase access to natural products for industrial applications, I have employed a metabolic engineering system for diterpenoid biosynthesis in recombinant bacteria. Here we utilize metabolic engineering to explore the potential for combinatorial biosynthesis, building on previous work from our group and others identifying consecutively acting diterpene cyclases and synthases. I have co-expressed different pairs of diterpene cyclase and synthase enzymes via our metabolic engineering system to characterize product yield. Specifically, 4 different diterpene synthases were each co-expressed with one of 20 different diterpene cyclases. We observed 10 previously unknown products which required purification and structural analysis via NMR. Our studies provide novel biosynthetic access to these 10 previously inaccessible diterpenes as well as increased yield to other diterpenoids, demonstrating the power of this combinatorial biosynthetic approach to provide availability to an array of structurally distinct natural products.

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Abstract 1913

Investigating the role of disulfide bonds in regulation of diatom violaxanthin de-epoxidase-like enzyme 1 (VDL1)

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In photosynthetic organisms, violaxanthin de-epoxidase (VDE) is activated by a pH decrease in the thylakoid lumen under excess light. In order to dissipate energy under high light conditions, VDE converts the energy-harvesting carotenoid violaxanthin to zeaxanthin, which is implicated in non-photochemical quenching. Here, we seek to understand the biochemical role of a VDE-like enzyme, VDL1, in the diatom *Thalassiosira pseudonana*. Like VDE, VDL1 has three domains: a cysteine-rich N-terminal domain, a lipocalin-like domain which serves as the catalytic center, and a negatively charged C-terminal domain. While it is well known that the cysteines of the N-terminal domain are essential for the VDE activity, the role of the disulfide bonds in VDL1 enzymes is not clear. We first attempted to investigate the turnover of putative carotenoid substrates violaxanthin, zeaxanthin, and diadinoxanthin with recombinant VDL1, with no observable product formed. Hence, in this study, we investigate the role of the disulfide bonds of the N- terminal domain through investigation of the isolated N-terminal domain. The N-terminal domain of a *T. pseudonana* VDL1 was generated recombinant expression in *E. coli*, and 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) was to quantify the number of oxidized versus reduced cysteines under a range of redox poise. Once we better understand how the structure of the N-terminal domain responds to redox poise, we hope to identify conditions that allow the N-terminal domain to activate the enzymatic activity of VDL1 *in vitro*.

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Abstract 1922

Plant-exclusive domain of trans-editing enzyme ProX-ala confers dimerization and enhanced tRNA binding

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Faithful translation of the genetic code is critical for the viability of all living organisms. Some aminoacyl-tRNA synthetases (aaRSs) are error-prone, mispairing noncognate amino acids with cognate tRNAs. Many aaRSs have evolved quality control mechanisms to prevent mistranslation. Bacterial prolyl-tRNA synthetases (ProRSs) mischarge noncognate Ala onto tRNAPro and possess an insertion (INS) domain that can deacylate this mischarged tRNA, thus avoiding mistranslation. Some bacteria and all eukaryotes lack an INS domain and instead, encode a free-standing trans-editing domain homolog, ProXp-ala. Sequence alignments revealed that plant ProXp-ala sequences contain a conserved C-terminal domain (CTD) that is absent in other organisms; the origin, structure, and function of this extra domain are unknown. To characterize the plant-specific CTD, we performed bioinformatics and computational analyses that were consistent with a conserved α-helical structure. We also expressed and purified wild-type *Arabidopsis thaliana (At)* ProXp-ala in *Escherichia coli*, as well as variants lacking the CTD or containing only the CTD. Circular dichroism spectroscopy confirmed a loss of α-helical signal intensity upon CTD truncation. Size-exclusion chromatography revealed that wild-type At ProXp-ala was primarily dimeric and CTD truncation abolished dimerization *in vitro*. Bimolecular fluorescence complementation assays in *At* protoplasts support a role for the CTD in homodimerization *in vivo*. The deacylation rate of Ala-tRNA^{Pro} by *At* ProXp-ala was significantly reduced in the absence of the CTD and kinetic assays indicated that the reduction in activity is primarily due to a tRNA binding defect. Overall, these results broaden our understanding of eukaryotic translational fidelity to include the plant kingdom. Our studies revealed that the plant-specific CTD plays a significant role in substrate binding and canonical editing function. Through its ability to facilitate protein-protein interactions, the CTD may also provide expanded functional potential to *trans*-editing enzymes in plants.

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ASBMB

Abstract 1927

Cloning and recombinant expression of a lactobacillus ferulic acid esterase for potential use in beer production

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Andrew Piefer

Ferulic acid (FA) is a precursor to 4-vinyl guaiacol (4VG), a clove-like flavoring component of Belgian-style and Bavarian wheat beers produced during fermentation. FA is released by ferulic acid esterases (FAEs), a class of enzymes that hydrolyze the ester bond between hydroxycinnamic acids and lignin in plant cell walls. This project aims to express, purify, and characterize a FAE from *Lactobacillus johnsonii* for possible use in brewing and distilling to release more FA during the mashing process. A codon-optimized FAE gene (LJ0536) was inserted into the expression plasmid pTXB1 (New England Biolabs), selected for its C-terminal self-cleaving intein and chitin-binding domain, which allows affinity purification using a chitin column. The expression plasmid was transformed into T7 Express competent *E. coli*, enabling inducible protein expression using IPTG. Preliminary expression experiments revealed high levels of FAE expression. We are currently optimizing protein expression for solubility by varying growth temperature, time, and IPTG concentration. Once the solubility is improved, we will proceed to purification and characterization. For characterization, the ideal pH and temperature for enzyme activity will be assayed by measuring the release of FA from a precursor polymer using HPLC. The results of this research will determine if recombinant *Lactobacillus johnsonii* FAE is effective for releasing FA from malt.

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Abstract 1935

Synthesis and Evaluation of Pterostilbene Amino Alcohol Derivatives as Antioxidants and Reversible Acetylcholinesterase Inhibitors

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Individuals with Alzheimer's disease (AD), one of the most common causes of dementia, display serious neurological deficits characterized by the decrease in various cognitive functions. The cause of AD is yet to be determined, but reversible inhibitors of acetylcholinesterase (AChE) have been shown to temporarily improve cognitive function in individuals with AD. The synthesis of new, multifunctional inhibitors for AChE is the necessary basis to improve currently available AD medications, possibly extending their duration of action. Five different pterostilbene amino alcohol derivatives were synthesized, as similar compounds have been shown to be reversible inhibitors specific to AChE. The novel derivatives were then characterized through NMR spectroscopy and evaluated in enzyme activity assays to determine their effectiveness as AChE inhibitors. Both secondary and primary amines were used in the synthesis, with primary amine derivatives showing significantly higher rates of inhibition (decrease of activity of AChE by 45–52%) than those containing secondary amines (decrease in activity by 10–15%). Besides their inhibitory ability, the additional antioxidant activity of pterostilbene and the alcohol group of the derivative can have a further neuroprotective effect against H2O2 and reactive oxygen species induced cell damage. The derivatives' antioxidant activity was evaluated using DPPH assays, with primary amine compounds also displaying significantly higher antioxidant activity than secondary amine compounds, increasing by almost 50%. Additionally, similar derivatives containing secondary amines have shown potential inhibitory activity on the self-induced aggregation of amyloid β_1–42, a protein fragment widely found in the brains of individuals with AD. Further investigations of these compounds in *in-vitro* assays are necessary to determine if the novel primary amine derivatives display similar effects. The data suggests that the synthesized compounds can be multifunctional inhibitors for AChE, making them a possible starting point for the development of new AD medications.

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Abstract 1937

A path toward the characterization of carboxyspermidine decarboxylase from two human gut microbes

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The dysregulated production of polyamines, such as spermidine, by human gut microbes is associated with disease states such as colon and pancreatic cancer. The biosynthesis of spermidine by human cells is well studied; however, human gut microbes produce spermidine through an alternative pathway that is less well characterized. The long-term goal of this research is the mechanistic characterization of carboxyspermidine decarboxylase which decarboxylates carboxyspermidine to produce spermidine in many gut microbes. We have developed a workflow for the heterologous expression, purification and crystallization of carboxyspermidine decarboxylase from *Clostridium leptum* (ClCASDC) and from *Bacteroides fragilis* (BfCASDC). A thermal shift assay was used to identify improved buffer conditions leading to crystallization and X-ray diffraction for ClCASDC. Expression in KRX cells and the addition of ion exchange chromatography were used to remove contaminating proteins from our initial BfCASDC nickel affinity purification from BL21 cells. To establish a kinetic assay, we are synthesizing the carboxyspermidine substrate using a method not present in the literature. Through this work we are developing the methods necessary for future structural studies using X-ray crystallography and mechanistic studies using steady-state and transient state kinetic methods. CASDC represents a potential drug target and these studies will provide the mechanistic basis for the future investigation of inhibitory compounds.

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Abstract 1940

Carboxyspermidine dehydrogenase of human gut microbe polyamine biosynthesis

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Polyamines are positively charged alkylamines found in almost all cells. Humans obtain them in three ways; dietary intake, metabolic production, or the uptake of polyamines made by microbes within the gut. Polyamines have wide-ranging functional roles in cell growth and proliferation and the dysregulation of microbe-produced polyamines has been implicated in conditions such as colon cancer, diabetes and obesity. The pathway most commonly used by gut microbes to produce polyamines differs from the human pathway. This alternative pathway uses carboxyspermidine dehydrogenase (CASDH), an enzyme without prior structural or functional characterization, to produce carboxyspermidine as a precursor to spermidine biosynthesis. We solved a 1.94 Å X-ray crystal structure of *Bacteroides fragilis* CASDH. This structure demonstrates the dimeric nature of BfCASDH, reveals the active site residues involved in catalysis, and leads us to propose updated functional annotations for two existing PDB entries. Steady-state kinetics were used to determine substrate specificity, kcat and kcat/Km parameters for BfCASDH and *Clostridium leptum* CASDH. Putrescine and diaminopropane substrates have similar kcat/Km values suggesting that carboxynorspermidine or carboxyspermidine are potential products. NADPH is the coenzyme as no catalysis was observed with NADH. Binding order was predicted, with NADPH binding fastest and aspartate semialdehyde preceding putrescine. These data provide the first steady-state kinetic and structural characterization of CASDH – a key enzyme in the production of polyamines by human gut microbes.

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Abstract 1942

Interactions that shape the complementary reactivities of flavins in bifurcating electron transferring flavoproteins

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Flavin-based electron transfer bifurcation accomplishes the feat of producing highly reducing (very negative E°) electrons at the expense of a less potent source. In bifurcating electron transferring flavoproteins (BfETFs) a pair of modestly reducing electrons is acquired from NADH, by a flavin (the so-called 'bifurcating flavin', Bf-flavin). Favorable transfer of one electron (1e) to a higher midpoint potential (E°) acceptor is mediated by a second flavin, called the 'electron transfer' (ET) flavin. However for the Bf-flavin, loss of this electron must produce a semiquinone that is high in energy, to reduce the other electron acceptor at very low E°. Thus, exergonic electron transfer to the ET-flavin must 'pay for' production of an energetic semiquinone (SQ) state which will be a transient species, not a thermodynamic product. Crucially, although the ET-flavin carries out 1e reactions at higher E°s, whereas the Bf-flavin must execute 2e chemistry at lower E°, they are chemically identical non-covalently bound FADs. The different reactivities of the two flavins stem from the relative energies of the different oxidation states they can adopt. For both, the options identified are oxidized (OX), anionic semiquinone (ASQ, attained upon acquisition of 1e by OX) and AHQ (attained upon acquisition of hydride = 2e- + H+ by OX). Consistent with its role, the ET-flavin has a stable ASQ state, although this is not the norm for flavin in water. In contrast, ASQ is only observed for the Bf-flavin under non-equilibrium conditions or when the BfETF has been perturbed by amino acid substitution or depletion of ET-flavin. Hence, interactions with the protein must stabilize ASQ in the ET site but destabilize it in the Bf site, relative to OX. We seek to understand how. To elucidate the roles individual residues and specific interactions in creating the energy landscape of each flavin, we are replacing residues that interact with each of the flavins and characterizing their effects on flavin redox energetics (via measurement of E°s) and electronic structure (via the optical signatures). We are complementing these measurements with quantum chemical calculations to learn how specific interactions between protein and flavin exert their effects. We have examined the significance of (1) electrostatics, (2) constraints on flavin geometry and (3) individual hydrogen bonds. Our computations succeed in replicating observed behavior, confirming their validity. The computations in turn allow us to learn about states that cannot be observed experimentally. Computed charge distributions make sense of the effects of amino acid substitutions, and provide a rationale for how the ET site can stabilize ASQ whereas the Bf site does not. Meanwhile, replacement of Arg residues near each of the flavins demonstrates that electrostatics tunes the E° of the ET-flavin, but plays a more important role in structural integrity in the Bf site. Thus, we are learning how individual interactions in each of the two sites can produce contrasting flavin chemistries even though some of the same amino acids are involved. Our work paves the way for modifying flavin sites to achieve desired reactivity.

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Abstract 1947

Two Human Gut Microbiome-Derived Enzymes that Reduce Prednisone and Other Corticosteroid Drugs

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Kailey Paar, Jackson Demartino

The gut microbiome is known to have various interactions with drugs and therapeutics, which can lead to varied health outcomes in individuals. However, the molecular mechanisms of drug metabolism by gut microbes have not received much attention and these reactions are still being discovered. To address this problem, we have targeted several proteins expressed by human gut microbes which are known to change the structure of the host-produced cortisol. We predicted that corticosteroids, a class of drugs that structurally mimic cortisol (e.g. prednisone, dexamethasone), may also be modified by known cortisol-modifying enzymes if they display substrate promiscuity. We have characterized two 20-hydroxysteroid dehydrogenases (20-HSDHs) with opposite stereospecificity from the gut microbes *Clostridium scindens* (20α-HSDH) and *Bifidobacterium adolescentis* (20β-HSDH). After the cortisol modification was verified, we have discovered that these enzymes can also modify corticosteroid drug compounds. Here we have found that the 20β-HSDH is kinetically more efficient and faster than the 20α-HSDH on all substrates, but each has a narrow substrate scope. Now that we have verified that these enzymes display some promiscuity in their substrate preferences, we are beginning to produce and characterize other known cortisol-modifying enzymes. This investigation aims to support the development of personalized medicine through predicting drug metabolism by knowing the makeup of an individual's microbiome.

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Abstract 1951

Establishing a Substrate Binding Assay using Desferrioxamine D Variant R306Q

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Katherine Hoffmann

Antibiotics are inhibitors targeted towards essential biological functions, functionally stopping the reproduction of the bacteria, or killing the bacteria outright. DesD, a nonribsomal-peptide-synthase-independent siderophore (NIS) synthase protein from the species *Streptomyces coelicolor*, catalyzes the last three steps in a pathway that makes a siderophore called desferrioxamine E. DesD is a drug target in our research because it is a model for other NIS synthase family members, which are present in some of the most virulent and pathological bacteria, like MRSA and Anthrax. Understanding DesD and designing an inhibitor for it will hopefully lead to a new class of antibiotics. Previous work in the Hoffmann Lab determined the structure, and kinetic turnover of wild-type DesD. This work revealed that wild-type DesD bound fully with the nucleotide substrate in the active site of the dimer. We propose a binding study to quantify the binding interaction with DesD, but will need to use variant R306Q, which showed catalytic inactivity when performing kinetic assay, to avoid catalysis. We hypothesize that the nucleotide will fully bind in both active sites of the dimer, but the substrate dfoG or HSC will bind with substrate in only half of the active sites (a cooperative effect). The key methods in determining the binding of R306Q are overexpression and purification of the protein, as well as isothermal titration calorimetry (ITC). The protein is overexpressed in *E. coli*, then purified using Affinity Chromatography. After these first two steps we use ITC to determine the binding thermodynamics in triplicate. We have performed binding assays with different concentrations of ATP and 10 mM of R306Q and got preliminary results confirming the results with the wild-type DesD. We have also begun the developing the binding conditions with the product desferrioxamine E limiting, along with ATP, and 10 mM of R306Q. Further research will be binding studies with substrate including desferrioxamine G and HSC with variant R306Q.

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ASBMB

Abstract 1960

Development of a LC-TQ-MS assay to examine functional diversity among metallophore-producing aminobutanoyl transferases

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Madeline Shoemaker, Jeff McFarlane

Transition metals such as iron are essential for living cells and must be acquired from the local environment. Under metal-limited conditions, some organisms biosynthesize and secrete metallophore natural products that capture metals prior to re-uptake by specialized membrane transporters. Nicotianamine-like metallophores (named for their discovery in the tobacco genus Nicotiana) use a S-adenosyl-L-methionine (SAM) dependent aminobutanoyl transferase (ABT) to form a backbone metallophore scaffold from aminobutanoyl moieties derived from SAM. In some cases, the scaffold is modified by the addition of amino acids or the alteration of functional groups adding potential ligands for chelation. This family of ABT enzymes have shared domain structures and sequence identity, but they perform varied reaction chemistries. Variations include the incorporation of one, two or three aminobutanoyl moieties into the scaffold or the addition of different amino acids. Existing knowledge does not allow the prediction of these variant chemistries from ABT sequences, presenting the need for an ABT assay capable of identifying possible products. Liquid chromatography in tandem with electrospray ionization triple quadrupole mass spectrometry (LC-TQ-MS) was used to develop an ABT assay based on the separation of SAM and the product methylthioadenosine (MTA). MTA was identified using positive polarity selective ion monitoring followed by collision induced dissociation and product ion selection. Initial method development used a carboxyazetidine-forming ABT from *Pseudomonas aeruginosa*. Current work is underway to apply this assay to identify unknown ABT metallophore products from different phylogenetic groups to establish the determinants of varied ABT reaction mechanisms.

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Abstract 1970

Mutation of Schizophyllum commune Type I Metacaspase for Cleavage Site Identification

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Metacaspases are cysteine proteases that are found in all non-metazoans. Metacaspases utilize a cysteine-histidine dyad to hydrolyze peptide bonds found after lysine and arginine residues that are recognized as cleavage locations. Many metacaspases are dependent on calcium for activation and undergo autoproteolysis in the presence of calcium ions. There are three different types of metacaspases, and our main study focuses on one of the Type I metacaspases of the fungus Schizophyllum commune (ScMCA-Ia). The study of metacaspases is difficult as the introduction of micromolar levels of calcium ions induces autoproteolysis and only small fragments of the original protein will be present. Therefore, our goal is to identify potential cleavage sites of ScMCA-Ia to purify intact protein. Previous research has characterized the autoproteolytic activity of the wild-type protein and potential lysine and/or arginine residues have been identified as cleavage sites. Several Lys and Arg residues have been mutated to Ala in an attempt to abolish cleavage at those positions. Mutation of the active site cysteine-histidine dyad is performed in tandem with lysine/arginine mutations to reduce the activity of ScMCA-Ia and prevent autoproteolysis. Proteolysis of the mutant proteins is analyzed by adding catalytic amounts of wild-type metacaspase protein, and proteolytic activity is monitored using an SDS-PAGE assay. Activity assay results confirm that mutation of the active site reduces the activity of ScMCA-Ia. The mutational analysis has narrowed the list of Lys and Arg that could be cleaved during the autoproteolysis of ScMCA-Ia. Several lysine residues have been found not to be sites of autoproteolysis, including K193, K197, K269, K305, and K375. Future work will focus on further identification of lysine and/or arginine residues that may play a role in autoproteolytic activity.

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Abstract 1978

Structural basis for the calmodulin-mediated activation of eukaryotic elongation factor 2 kinase

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The highly energy-consuming process of translating mRNA into functional proteins occurs at the ribosome in 3-steps: initiation, elongation, and termination. The protein translation rate is tightly regulated and is essential in processes such as protein folding and transit, energy/nutrient adaptation, and memory formation. Eukaryotic elongation factor 2 kinase (eEF2K), a calmodulin-dependent α-kinase, regulates protein elongation through a phosphorylation event on eEF2, reducing eEF2's affinity for the ribosome, thus suppressing protein elongation. Although aberrant eEF2K activity is associated with chronic diseases, such as neurodegenerative disorders and tumorigenesis, the structure of eEF2K has not been resolved. The lack of structural information presents challenges in determining the mechanism of activation, regulation, and development of novel therapeutics. Here, we examine the mechanism of eEF2K activation and regulation in the context of our 2.3-Å crystal structure of calmodulin in complex with a functional truncated construct of eEF2K (eEF2KTR). This construct made crystallization of an active complex possible by excluding disordered regions while conserving enzyme function, demonstrating similar activation by calmodulin and activity towards eEF2 as the wild-type enzyme. Our structural data indicate that the C-lobe of calmodulin is intimately associated with the N-terminus of the kinase and plays a central role in activating the enzyme. Structural data, in conjunction with previous and current biochemical and biophysical studies, suggest that upon binding to the calmodulin-targeting motif at the N-terminus of eEF2K, calmodulin creates an "activation spine" that communicates with the active site through a highly conserved regulatory element. Using structural information, we predicted residues within the activation spine of eEF2K that would be critical for calmodulin binding and activation. We used mutational analysis by steady-state kinetics to probe specific functions. Additional crystallographic and steady-state kinetic data have led to the discovery of a unique allosteric regulation mechanism. This study reveals novel structural information on the heterodimeric complex of calmodulin with eEF2KTR, which can be utilized for further investigation of eEF2K's mechanism of activation and regulation.

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Abstract 1982

Expression and characterization of an atypical metallocarboxypeptidase from *Agaricus bisporus*

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Faith Kaluba, Zoe Oster

In order to catalyze a chemical reaction, an enzyme active site requires specific amino acids in specific locations. The active site of a carboxypeptidase, which cleaves C-terminal amino acids from substrate proteins, has well-defined components which are disrupted in pseudoenzyme members. A study of the fungal pseudocarboxypeptidase, Ecm14, discovered basidiomycete homologs in which most required active site amino acids were present but locations were apparently swapped. In order to determine if these Ecm14-like proteins exhibited enzymatic activity, one such protein from *Agaricus bisporus* was modeled and expressed in HEK293T and Sf9 cells. Models predicted several unique loops and proprotein convertase cleavage sites. Expression in HEK293T cells was primarily intracellular, while the protein was secreted from Sf9 cells. After incubation with chymotrypsin to remove the prodomain, enzymatic cleavage of C-terminal phenylalanine was detected, albeit weakly. Purification of this putative enzyme is underway. This research suggests that the specific arrangement of amino acids in the active site of an enzyme may be flexible. This also suggests that other proposed pseudoenzymes could actually have enzymatic function despite a rearrangement of active site amino acids.

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Abstract 1992

Revealing the secrets of metalloenzymes one snapshot at a time

Catherine Drennan, *MIT and HHMI*

How do microbes live on the pollutant carbon monoxide? How do microbes split the triple bond of nitrogen gas? When it comes to performing difficult chemistry, microbes often combine a protein scaffold with a highly reactive metallocofactor, employing a hired gun, if you will. The Drennan lab combines X-ray crystallography and cryo-electron microscopy with other biophysical methods with the goal of revealing the secrets behind metalloenzyme chemistry. In her Rose Award presentation, Drennan will present her lab's structural studies of ribonucleotide reductases (RNRs). RNRs employ metallocofactors to generate radical species to afford the conversion of ribonucleotides (the building blocks of RNA) to deoxyribonucleotides (the building blocks of DNA). These enzymes are chemotherapeutic targets and proposed antibacterial targets. Here, she will describe how the "resolution revolution" of cryo-electron microscopy allowed for the capture of an active state structure of ribonucleotide reductase for the first time.

This research was supported in part by NIH. CLD is an HHMI Investigator.

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Abstract 1995

Functional Roles of Conformational Dynamics in Deubiquitinating Enzymes

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Attachment of ubiquitin to substrate proteins, namely ubiquitination, is an important form of posttranslational modifications that controls many biological processes in eukaryotes, such as protein degradation, DNA damage responses, cell proliferation, and immune responses. Deubiquitinating enzymes are proteases that cleave monoubiquitin or polyubiquitin chains from substrate proteins, thereby modulating their stability, activity or interaction specificity. Conformational flexibility allows enzymes to sample many sub-conformational states, some of which are functionally important. Numerous structural and biochemical studies suggest that deubiquitinating enzymes possess conformational flexibility essential for catalytic turnover and substrate specificity. However, the exact functional roles of conformational dynamics are poorly defined partly due to the lack of detailed information on the conformational substrates and the rates of interconversion between these states. Our recent NMR study on a deubiquitinating enzyme, debuiquitinase A (DUBA)/OTUD5, characterized motions on the sub-millisecond timescale in DUBA. We found that many structural elements in DUBA transition into a sparsely populated conformational state in a global conformational exchange process. By combining NMR relaxation dispersion experiments, enzyme kinetics and site-directed mutagenesis, we identified residues that control this conformational exchange process and confirmed the functional importance of these residues. More importantly, we elucidated the molecular mechanism by which DUBA is activated by phosphorylation of a serine residue, Ser177, located at the beginning of the α1 helix. The interaction between the phosphorylated Ser177 and a positively charged structural element, α6 helix, allows the motion of the α1 helix to be coupled to the global conformational exchange process. In the nonphosphorylated (inactive) form of DUBA, Ser177 undergoes motions faster than the global conformational exchange process, suggesting that the coupled motions of α1 helix and the rest of DUBA in the phosphorylated (active) form are essential for activity. In other words, DUBA is activated by subtle changes in the dynamic properties of key residues rather than significant structural changes. Our data also suggest that the transient unfolding of the α6 helix drives the transition into the sparsely populated state. We have recently extended our study to a deubiquitinating enzyme, OTUD3, which belongs to the same family as DUBA. We detected a similar global conformational exchange process in OTUD3 by NMR relaxation dispersion experiments. Comparison of wildtype and mutant forms of OTUD3 with lower activity support the functional importance of this conformational process in OTUD3. Overall, our study suggest that the conformational exchange process observed on OTUD5 and OTUD3 is

functionally important and may be conserved across members of the ovarian tumor (OTU) family of deubiquitinating enzymes. Given the fact that deubiquitinating enzymes are an emerging class of therapeutic targets, our study may benefit the design of therapeutic strategies to modulate the function of deubiquitinating enzymes for treating various diseases, including cancer, neurodegeneration, and inflammatory diseases.

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Abstract 2011

A Role for Serine 26 in the Folate Half Reaction of *E. coli* Methylenetetrahydrofolate Reductase (MTHFR)

Benjamin Brim, *Grinnell College*

Yuwei Pan, Richard Li, Maxwell Tetrick, Elizabeth Trimmer

Methylenetetrahydrofolate reductase (MTHFR) is a flavin-dependent enzyme involved in folate metabolism. It converts 5,10-methylenetetrahydrofolate (CH2-H4folate) to 5-methyl tetrahydrofolate (CH3-H4folate). In human MTHFR, mutations have been linked to cardiovascular disease and neural tube defects. Here, we aim to characterize *E. coli* MTHFR which acts as a model for the catalytic domain of the human enzyme. Its overall reaction can be split into two separate half-reactions, a reductive half-reaction where NADH is oxidized to reduce the enzyme-flavin complex and an oxidative half-reaction where CH2-H4folate is reduced to CH3-H4folate. For the oxidative half-reaction, we propose that protonation at N10 of CH2-H4folate leads to a 5-iminium cation intermediate which undergoes reduction by the reduced flavin. Near where folate binds in the active site, Glu28, His273, and Ser26 participate in a hydrogen bonding chain, and we hypothesize that these three residues function in a proton relay mechanism to create the 5-iminium cation intermediate in the oxidative half-reaction. Previous site-directed mutagenesis studies have shown Glu28 to be essential for the oxidative half-reaction and important in the reductive half-reaction, and His273 has been shown to be important in the oxidative half-reaction. Using Ser26Thr, Ser26Ala, and Ser26Val mutant enzymes, we aimed to probe the importance of Ser26 in both half reactions. This was done through two steady-state kinetic assays which use menadione as an alternate electron acceptor to reoxidize the flavin: a CH3-H4folate/menadione oxidoreductase assay where the reductive half-reaction with CH3-H4folate is rate limiting and a NADH/menadione oxidoreductase assay where the reductive half-reaction with NADH is rate limiting. In the CH3-H4folate /menadione assay, the Ser26Thr and Ser26Ala mutant enzymes showed less than a two-fold change in kcat whereas the Ser26Val mutant enzyme showed a ten-fold decrease in kcat compared to the wild-type (WT) enzyme. For the NADH/menadione assay, all variants showed comparable activity to the WT enzyme. These results suggest an importance for Ser26 in *E. coli* MTHFR, specifically within the oxidative half-reaction. They are also consistent with the participation of Ser26 in a proton relay catalyzing the oxidative half-reaction.

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Abstract 2016

Utilizing a single rSAM RiPP maturase for generalizable and predictable crosslink formation in a wide array peptide substrates

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Andrew Roberts, Vahe Bandarian

Enzymatic transformations that functionalize unactivated C-H and C-C bonds are of considerable contemporary interest. However, the ability to harness the potential of an active site for directed modifications is generally limited due to the exquisite substrate specificities that are hallmark of enzyme catalysis. In the context of reactions at unactivated centers, members of the growing class of enzymes in the radical S-adenosyl-L-methionine (rSAM) superfamily are enticing because of their ability to activate carbon atoms by H-atom abstraction to access unprecedented transformations that generate complex natural products. These rSAM enzymes bind SAM to the unique iron of a [4Fe-4S] cluster, which is held in the active site by interaction with Cys thiolate sidechains of residues in a conserved CxxxCxxC motif. The reductive cleavage of SAM generates a 5′-deoxyadenosyl radical, which initiates substrate activation by H-atom transfer. The growing repertoire of reactions attributed to rSAM enzymes underscore their potential in biotechnology applications. However, the ability to leverage rSAM maturase catalytic prowess towards chemistry on non-native substrates is limited by their generally high substrate specificity. This presentation will highlight our studies on the promiscuity of a single rSAM maturase, which catalyzes the introduction of a thioether crosslink in a ribosomally encoded and posttranslationally modified polypeptide (RiPP). Our biochemical data probing substrate tolerance have utilized a wide array of peptide sequences—we see efficient crosslink installation in sequences that range from slight modifications in the native peptide to completely non-natural sequences including those that utilize unnatural amino acids. The results of these observations have led to rules that allow us to predict reactivity of the enzyme against novel peptides. The insights from our substrate tolerance studies are leveraged to produce several analogs of FDA-approved peptide therapeutics, where the physiologically unstable disulfide is replaced with a thioether crosslink. This work underscores the potential use of this enzyme in biotechnology applications and provides a previously underexplored path for development of rSAM enzymes as tools in the synthesis of chemically challenging and physiologically stable peptide scaffolds.

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Abstract 2045

Identification of structural regions contributing to deamination-independent antiviral function in APOBEC3G, a cell-innate defense against HIV

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Tianci Guan, Ann Sheehy

APOBEC3G (A3G) is a cellular restriction factor that suppresses the proliferation of Human Immunodeficiency Virus (HIV). The protein short circuits viral infection via mutations introduced by a characterized cytidine-deaminase activity and still undefined deamination-independent function. A comprehensive structure-function analysis was undertaken to identify specific protein domains contributing to deaminase-independent viral restriction. A library of variants (n ∼135) has been generated utilizing a near-saturating alanine scanning mutagenesis approach. The catalytic and antiviral activities of variants are assessed using independent functional assays: an antibiotic resistance assay was used to assess enzymatic function, and a reporter cell infectivity assay characterizes anti-viral activity. Variants exhibiting loss of antiviral activity without significant reduction in catalysis are further investigated to interrogate regions contributing to the protein's catalysis-independent activity. Preliminary results suggest that the regions within the N-terminus of A3G are important for deamination-independent HIV restriction. Alterations within the C-terminus more often result in variants with loss of catalysis, but maintenance in overall antiviral activity, suggesting that the deamination-independent activity is unaffected by these amino acid changes. This suggests these residues are not as critical for catalysis-independent restriction. Identification of these critical regions can guide future structural analysis of A3G and identify regions of interest for future functional studies, elucidating the less well-defined mechanisms of anti-retroviral suppression by A3G.

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Abstract 2062

Importance of Leucine 277 for NADH Binding in Methylenetetrahydrofolate Reductase

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Junmin Hua, Tin Tran, Jason Chien, Elizabeth Trimmer

E. coli methylenetetrahydrofolate reductase (MTHFR) is an enzyme that reduces 5,10-methylenetetrahydrofolate (CH2-H4folate) to 5-methyltetrahydrofolate (CH3-H4folate) using nicotinamide adenine dinucleotide (NADH) and a bound flavin adenine dinucleotide (FAD). MTHFR catalyzes the reaction using a ping-pong Bi Bi kinetic mechanism, and the reaction can be broken into reductive and oxidative half-reactions involving NADH and CH2-H4folate, respectively. The Leu277 residue functions, along with a Leu212, Phe223, and Phe184, within the active site as a part of a hydrophobic pocket which binds each substrate using hydrophobic interactions and stacking. This study investigates the importance of Leu277 on the ability of MTHFR to bind its folate and NADH substrates. Due to the Leu277 residue proximity and orientation as compared to the binding location of each substrate, we hypothesized that Leu277 would impact both NADH and folate binding. However, the closer location of Leu277 to the folate substrate led to our prediction of a stronger influence on folate binding than NADH binding. The MTHFR mutants of interest are Leu277Ala, Leu277Ile, and Leu277Tyr. The alanine mutant was designed to be nonfunctional, the isoleucine functional, and the tyrosine was created to add aromaticity to the residue. The mutant enzymes were studied using two steady-state kinetic assays, both of which use menadione as an alternate electron acceptor to reoxidize the flavin: a CH3-H4folate/menadione oxidoreductase assay where the reductive half-reaction with CH3-H4folate is rate limiting and a NADH/menadione oxidoreductase assay where the reductive half-reaction with NADH is rate limiting. In the NADH-menadione assay, we found that the Leu277Ala mutation increases the Km for NADH 7-fold, but did not affect kcat as compared to the wild-type enzyme literature values. The Leu277Ile mutant showed no significant change in either the kcat or Km for the NADH-menadione assay. The Leu277Tyr mutation increased the Km for NADH by 22-fold while not statistically affecting the kcat of the reaction. In the CH3-H4folate-menadione assay, we found that the Leu277Ala and Leu277Tyr mutant enzymes showed a 2–3 fold decrease and increase, respectively, in the kcat. Leu277Ala demonstrated no change in Km for CH3-H4folate, while Leu277Ile showed a small decrease. In summary, the Leu277 residue appears to play a significant role in the binding of NADH, but not in catalysis of the reductive half-reaction. Contrary to our hypothesis, Leu277 seems to not participate in folate binding; it may have a small role in folate catalysis.

Grinnell College.

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Abstract 2087

Exploring the role of the dimer interface in *Plasmodium falciparum* malate dehydrogenase: The impact of Q11I, I15Q, L19N and L22N mutations on quaternary structure and enzymatic properties

Daniel Armendariz, *University of San Diego*

Diego Hernandez, Megan Keene, Jessica Bell, Ellis Bell

Many enzymes show behavior suggesting allosteric interactions involving communication between subunits in a quaternary structure. Malate Dehydrogenases from a wide variety of organisms have either a dimer or a tetrameric quaternary structure, and subunit interactions have been suggested to be involved in both activity and regulation of these enzymes. Despite the potential importance of understanding the molecular mechanisms of such subunit interactions in allosteric drug design, little is known at the molecular level of how the subunits in malate dehydrogenase communicate the presence of a ligand on one subunit to another subunit in the quaternary structure. *Plasmodium falciparum* (the parasite mainly responsible for malaria has a tetrameric malate dehydrogenase that consists of four identical polypeptide chains in a dimer of dimer type structure with three potential types of subunit interface- an A-B interface, an A-C interface, and an A-D interface. The A-B interface resembles that found in dimeric malate dehydrogenases. Analysis of the dimer A-B interface shows a unique feature in the first region of the multipart interface, the existence of a clade conserved Glutamate residue, E18, in the middle of an alpha helix that makes contact with the equivalent helix across the interface. MM-GBSA analysis using HawkDock suggests that E18 interactions across the interface are antagonistic. We hypothesize that the polarity of the local environment of E18, governed by 4 conserved residues Q11, I15, L19, and L22 in *Plasmodium falciparum* Malate Dehydrogenase modulates the interactions E18 makes across the interface and helps govern ligand-induced subunit interactions necessary for the normal function of the enzyme. To test this hypothesis, we constructed site-directed mutants of each of these four residues Q11I, I15Q, L19N, and L22N. Mutants were sequence confirmed, and the resultant proteins were expressed, purified using nickel-NTA affinity chromatography, and characterized using enzyme kinetics and size exclusion chromatography. All four mutants had significantly lower specific activities than the wildtype enzyme, with Q11I having the highest turnover number of the mutants, approximately 8% of the wildtype. Size exclusion chromatography showed that the Q11I mutation caused a shift in quaternary structure from a simple tetramer structure to a tetramer-dimer-monomer equilibrium. The L19N mutation shifts the equilibrium almost entirely to the monomer with minimal indication of tetramer or dimer forms. Homology models of the various mutants were constructed using PyMol mutagenesis and refined using GalaxyRefine Complex. The resultant models were then examined using the HawkDock

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MM-GBSA routine to explore the contribution of interface residues to the overall stability of the tetramer. Compared to the wildtype controls, Q11N had the least impact on the various tetramer interfaces while I15Q appeared to significantly strengthen the A-B interface (deltaGo = −176.1+/−1.7 kCals/mol vs wildtype = −165.2+/−1.5 kCals/mol) but had the weakest A-D interface. Overall, these results support our hypothesis concerning the importance of the local polarity around the conserved E18 residue in *Plasmodium falciparum* MDH, and form the basis for future studies aimed at exploring possible targets on *Plasmodium falciparum* MDH for potential drug design as well as providing basic information about the mechanisms of subunit interactions in oligomeric proteins.

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Abstract 2096

Biochemical characterization of protein 3r8e as a novel glucose kinase

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Michel Evertsen, Bonnie Hall

The Protein Data Bank (PDB) contains over 198,000 experimentally determined protein structures, approximately 4300 of which have not been assigned a specific function. One such protein is PDB ID 3r8e from *Cytophaga hutchisonii*, a potential kinase with a solved structure but no confirmed function. Utilizing modules from the Biochemistry Authentic Scientific Inquiry Laboratory (BASIL) consortium, we first used a range of in silico tools to analyze 3r8e. These online tools included BLASTp, Pfam, and DALI. Together the in silico results indicated that 3r8e could be a glucose kinase. Next, molecular docking was used to explore whether glucose was an appropriate substrate for 3r8e. Glucose and five other sugars were docked into 3r8e along with ATP. Although all the sugars could be docked, glucose had the best fit for the active site. The 3r8e protein was then overexpressed in *E. coli* and purified using nickel affinity chromatography. Protein purity was assessed using SDS PAGE analysis. The purified protein was subsequently used in coupled kinase assays to determine the specific activity of 3r8e for the six different sugar substrates. A high specific activity was seen for 3r8e with glucose as the kinase substrate. Little to no activity was seen using fructose, galactose, lactose, ribose, or sucrose. In summary, online tools, molecular docking, and coupled kinase assays using purified protein demonstrate that 3r8e is a glucose kinase.

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Abstract 2097

Using computational and biophysical approaches to explore knock in and knock out mutations of Ignicoccus islandicus and watermelon glyoxysomal malate dehydrogenases

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Jessica Bell, Ellis Bell

All Malate Dehydrogenases (MDH) have quaternary structure, many being dimeric with a single dimer subunit interface, while some are tetrameric in a dimer of dimers structure with both dimer interfaces and potentially two "tetramer" interfaces. Subunit interactions have been invoked in both catalysis and regulation, yet no studies have investigated the roles of specific residues or regions of the interfaces in such phenomena. Understanding the mechanisms of these interactions is an essential component of designing allosteric drugs that target malate dehydrogenase. We hypothesize that interactions at different regions of the dimer interface play critical roles in substrate saturation and regulation. Using the tetrameric Ignicoccus islandicus (IgIsl-MDH) and dimeric watermelon glyoxysomal (wm-g-MDH) forms, which have different kinetic properties, we have explored the second and fourth regions of the dimeric interface using a knock in, knock out approach. We switch discrete residues or regions of the dimeric interface and explore the impact in both wet lab and computational experiments. 4 knock in mutations have been studied: IgIsl KHAA to wm-g SHMD, IgIsl ATIW to wm-g SATL, IgIsl A54 to wm-g D92, and IgIsl A236 to wm-g Y273. Models were constructed in PyMol, using the IgIsl structure (6qss.pdb) and the wm-g structure (1smk.pdb), refined using Galaxy Refine Complex, and interface contributions explored using HawkDock MM/GBSA. The data showed the SATL knock in mutation had the largest effect on interface stability. Targeted SwissDock ligand docking showed the SATL knock in increased affinity for Pyruvate, Oxaloacetate and Citrate while the SHMD knock in decreased Citrate affinity. Reciprocal experiments where the IgIsl sequences were knocked into wm-g-MDH showed the dimer interface interactions in wm-g-MDH were significantly increased in the mutant where the IgIsl-MDH sequence ATIW was knocked in (162.11 +/−4.53 kCals/Mol to −177.67+/−2.68 kCals/Mol). A major effect on Citrate affinity (−8.91+/−0.03 kCals/Mol to −10.78+/−0.08 kCals/Mol) was observed, with minimal effects on Pyruvate or Oxaloacetate binding. In wet lab experiments, Size Exclusion Chromatography showed that the SATL knock in mutation had no effect on the tetrameric quaternary structure of IgIsl MDH. The IgIsl-MDH contains two tryptophans per subunit-Protein excitation and emission spectra show tryptophans have a significant blue shift to 328 nm indicating a distinctly hydrophobic environment which was used to explore protein stability in guanidine hydrochloride unfolding experiments for the IgIsl knock in mutants. Direct observation was compared with DeepDDG predictions. NADH fluorescence free in solution or bound to the IgIsl protein showed a distinct blue shift (430 nm) relative to free NADH (455 nm) when excited at 340 nm, as well as an 3-fold NADH fluorescence enhancement which was used to compare the binding of NADH to wildtype and the knock in mutations of IgIsl-MDH. Fluorescence spectroscopy also showed that the IgIsl MDH tryptophans form a resonance energy transfer couple to the reduced nicotinamide ring of the bound cofactor, providing another probe of mutant structural integrity. Overall this work provides support for the concept that different regions of the dimer interface are involved in activity and regulation of Malate Dehydrogenases and provides a structural understanding necessary for future development of potential allosteric drugs targeting Malate Dehydrogenase.

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Abstract 2102

Defining the role of a conserved motif at the interface of the substrate and FAD binding sites in FAD dynamics in class A flavin monooxygenases: a case study of 6-hydroxynicotinate-3-monooxygenase

Sam Belsky, *The College of Wooster*

Sipara Semu, Mark Snider, Zachary Turlington, Katherine Hicks

Understanding the mechanisms that initiate catalysis upon the binding of a substrate by an enzyme is critical for altering an enzyme's specificity or in the design of catalysts to produce novel products. Class A flavin monooxygenases catalyze the regiospecific hydroxylation of aromatic substrates in a process that requires elegant coordination of multiple phases of the reaction including substrate and NAD(P)H binding, flavin reduction by NAD(P)H, oxygen-adduct formation, substrate hydroxylation and product release. As a part of this class, 6-hydroxynicotinate-3-monooxygenase (NicC) catalyzes the decarboxylative hydroxylation of 6-hydroxynicotinate, converting it into 2,5-dihydroxypyridine, in the degradation of nicotinic acid by aerobic bacteria. An analysis of the structures of related class A monooxygenases has revealed a conserved motif found at the interface of the FAD and substrate binding sites which we've hypothesized could be important in inducing the conformational changes in FAD upon the binding of substrate. FAD conformations within this enzyme family are observed in an IN conformation where the isoalloxazine is proximal to the substrate, and in an OUT conformation when the isoalloxazine is in position to accept hydride from NADH after substrate binds. To interrogate whether this motif is important in the mechanism of controlling these IN/OUT conformations of FAD, variants of the conserved residues within this motif (namely G43P, A44G, G45A) and the substrate binding residues (H47Q and H47E) in NicC have been generated. Results from steady-state kinetic analysis show that the G43P and H47Q NicC variants demonstrate inhibition by 6-HNA, unlike what has been observed for the WT enzyme, suggesting that the shared binding domain for NADH and 6-HNA may not undergo the correct conformational change upon binding 6-HNA. Further analyses of the binding of 6-HNA by the G43P NicC variant by isothermal titration calorimetry, titration by UV changes, and rapid mixing kinetics by stopped-flow spectroscopy all indicate that the variant's affinity for 6-HNA has greatly increased in comparison to WT NicC. Moreover, the G43P NicC variant's ability to reduce the FAD, as analyzed by anaerobic stopped-flow spectrophotometry, is >100-fold slower than that of WT NicC. Despite these substantial changes in activity, the crystal structure of the G43P variant is virtually unchanged from the WT enzyme; both structures show FAD bound in the same orientation in the absence of 6-HNA. These observations suggest that the differences in activity are the result of changes in protein dynamics, supporting the hypothesis that the identified conserved motif plays a role in coordinating dynamics of flavin movement during catalysis in class A monooxygenases.

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Abstract 2108

Excess Manganese Increases Photosynthetic Activity via Enhanced Reducing Center and Antenna Plasticity in *Chlorella vulgaris*

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Amanda Smythers, Jessica Crislip, Danielle Slone, Derrick Kolling

The photosynthetic electron transport chain (ETC) relies on multiple redox active transition metals in metalloenzymes. One particularly important transition metal in this system is manganese which is an essential cofactor in photosystem II (PSII) and a component of the oxygen evolving complex (OEC). Further, manganese is also a component of antioxidants such as superoxide dismutases present in the membrane of chloroplasts, thus demonstrating its integral role in shaping the redox environment of chloroplasts. Interestingly, previous work has demonstrated that excess manganese exposure enhances photosynthetic activity rather than acting in a deleterious fashion (as is the case for exposure to an excess of other transition metals). This study quantified chlorophyll fluorescence, PSII abundance, reducing site activity, and antenna size and distribution to investigate the influence of manganese concentrations on the photosynthetic performance and PSII plasticity of the model alga *Chlorella vulgaris*. Overall, this study finds that excess manganese exposure induces an increase in PSII plasticity that is characterized by increases in photosynthetic activity and reducing center activity as well as reductions in antenna size and the proportion of PSIIb centers. These results suggest that manganese may be a key factor in enhancing photosynthesis beyond what is observed under standard conditions.

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Abstract 2111

Using enzyme kinetics and computational cocking studies to understand substrate and inhibitor interactions with human mitochondrial, human cytosolic, watermelon glyoxysomal and *Plasmodium falciparum* malate dehydrogenases

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Jessica Bell, Ellis Bell

Recently there has been increased interest in structure-function relationships in Malate Dehydrogenases (MDHs) fueled by oncometabolites (such as 2-Hydroxyglutarate) or drug design with pathogen MDHs as target. Differing metabolic roles of isoenzymes (e.g: human cytosolic vs mitochondrial), make understanding unique structural relationships that govern substrate specificity and regulator binding a challenge complicated by the wide range of conditions that result in substrate inhibition. Three arginine residues (R124, R139 and R196) using watermelon glyoxysomal MDH as reference, conserved in all MDHs, have been computationally mutated to either alanine or glutamine and their impact on oxaloacetate affinity (assessed using SwissDock) explored. To define the nature of active site interactions with ligands we also explored 19 other ligands including 2-to-5 carbon compounds and the allosteric inhibitor citrate. Mono and dicarboxylic acid ligands at each chain length were used and included various 2 position substituents. For selected substrates and potential inhibitors we have examined the ability to exhibit "substrate inhibition" or impact the normal activity of each enzyme in initial rate kinetics studies at both pH 8 and pH 7. We observed distinct differences in initial rate kinetics in oxaloacetate varied experiments. Human mitochondrial MDH showed the lowest Km values for oxaloacetate (8.9 μM, pH 8 and 7 μM, pH7) compared to human cytosolic MDH (27.4 μM, pH 8 and 13.6 μM, pH 7) or the *Plasmodium falciparum* enzyme (226 μM, pH 8 and 12.3 μM, pH 7). Both human isoforms showed significant substrate inhibition at high oxaloacetate concentrations with Ki values of 852 μM (cytosolic) and 524 μM (mitochondrial) at pH 8. The *Plasmodium falciparum* enzyme, like the watermelon glyoxysomal enzyme, did not exhibit substrate inhibition over the oxaloacetate concentration ranges used (0–2 mM). Vmax values for the enzymes gave turnover numbers of 484 & 514 at pH 8 and 7 respectively for human mitochondrial MDH, compared with 185 & 180 for human cytosolic and 23 & 63 for *Plasmodium falciparum*- all at pH 8 & 7 respectively. Computational docking studies indicated R124 mutations had the largest effect with the alanine mutant having a significantly greater impact than the glutamine mutant (ΔG° for wildtype = −9.5+/−0.05 kCal/mol, R124A = 6.4+/−0.02 kCal/mol, and R124Q = −7.04+/−0.01 kCal/mol). R130 and R196 mutations had lower impact with little difference between alanine and glutamine mutants suggesting that while R124 provides most of

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the binding interactions, R130 and R196 may be involved in correct alignment in the active site for catalysis. Human mitochondrial and *Plasmodium falciparum* MDH had much lower ΔG° for oxaloacetate binding (ΔG° = −11.05+/−0.04 and −10.85+/−0.08 kCal/mol respectively). The three-carbon substrate pyruvate had a lower ΔG° value, −8.12 +/−0.04 for human mitochondrial MDH, similar values were found for all isoenzymes studied. Docking studies suggest that *Plasmodium falciparum* and human mitochondrial isoforms had the highest affinity for citrate. Consistent with this pattern the *Plasmodium falciparum* enzyme tended to show higher affinity for 4–6 carbon analogs but similar affinity for 2–3 carbon compounds. This combination of wet lab experimental and computational data provides a solid basis for further exploration of structure-function studies of the active site region of malate dehydrogenases.

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Abstract 2136

Investigating reduction triggered conformational changes in the bifurcating electron transfer flavoprotein from *Acidaminococcus fermentans* by small angle neutron scattering

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Bifurcating electron transfer flavoproteins (Bf-ETFs) have garnered attention recently due to their ability to drive energetically uphill electron transfers. In order to replicate this remarkable feat in man-made energy-transducing materials and devices, we are working to understand how it is achieved in Bf-ETFs. Bf-ETFs are composed of two subunits (L and S, for large and small) and contain two FADs. The so-called 'head' domain is derived primarily from the L subunit and contains the ET- flavin, whereas the 'base' domain contains the Bf-flavin buried between subdomains derived from each of the L and S subunits. In the B-like conformation of Bf-ETF captured in crystals of *Acidaminococcus fermentans* Bf-ETF (AfeETF), the orientation of the head domain places the two flavins almost close enough for direct electron transfer (18 Å). However, in crystals Bf-ETFs from other species, the head domain is rotated by 80° placing the flavins 41 Å apart. Thus, rotation of the head domain can sever electrontransfer between the two flavins and is therefore proposed to gate electron transfer within Bf-ETF. We have used small angle neutron scattering (SANS) to study the solution structure of the AfeETF. Our goals were to establish the resting conformation of the AfeETF in the solution, and to determine whether a conformational change accompanies reduction of oxidized AfeETF to fully reduced AfeETF produced by reaction with its substrate NADH. We also tested whether such a change could be attributable to reduction per se (e.g., produced by reaction with dithionite) or to association with the nicotinamide. By determining whether the published crystalstructures can explain the scattering of the AfeETF we are also assessing the extent to which ETF populates multiple conformations in solution, as opposed to the fixed homogeneous states portrayed in crystal structures. SANS is ideally suited to these tasks. It is one of very few methods that does not perturb the oxidation states of flavins, because it does not employ photons. SANS was performed at the CG-3 Bio-SANS instrument at the Oak Ridge National Laboratory. Three different reduced samples were compared to assess the significance of oxidation state alone, binding to NADH, and/or complex formation with product NAD+. Non-perturbation of the sample oxidation states was confirmed via optical spectroscopy. Guinnear analyses documented well-behaved proteins and negligible aggregation in all cases. However, the pairwise distribution functions revealed poor agreement with theoretical predictions based on either of the conformations that have been crystalized. Thus, our data show that a significant population of ETF adopts a more extended conformation in

solution, or that portions of the protein are less structured in solution than in crystals. Moreover, this was oxidation state-dependent, suggesting a redox-coupled conformation change. The oxidized state displayed a larger radius of gyration (Rg) of 27.8 ± 0.1 Å and maximum dimension (Dmax) of 89 Å. compared to the values of Rg of 25.9 ± 0.1 Å. and Dmax of 82 Å, that best describe the set of reduced states. Thus, we have evidence of redox-coupled conformation change. Our high-quality data position us to perform ab initio modeling of the SANS data to obtain the conformations of the ETF in solution by sampling multiple conformations of the ETF based on the crystal structures, for the different states of the system.

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Abstract 2142

From negative to no cooperativity: Alteration of inner-subunit communication within F420H2:NADP+ Oxidoreductase

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F420H2:NADP+ Oxidoreductase (Fno) catalyzes the reversible reduction of NADP+ to NADPH, using reduced F420 cofactor as the hydride donor. NADPH production as well as oxidized F420 cofactor are linked to several metabolic pathways including glycolysis and methanogenesis within methanogenic and sulfate-reducing archaea. There are several enzymes that utilize F420 as a cofactor. Our work has provided valuable and new mechanistic insight into the enzymes that use this unique cofactor. For example, our previous pre steady-state kinetic studies on Fno, indicated biphasic kinetics with an initial burst followed by a subsequent slow phase, where the amplitude of the burst phase corresponds to only 50% of cofactor reduction. This indicates half-site reactivity where only one of the active sites are functional at a time within the dimer. The steady-state data suggests that Fno participates in negative cooperativity kinetics. The half-site reactivity and negative cooperativity phenomena observed within Fno suggests that the enzyme regulates NADPH production within the cell and therefore, participate in inner subunit communications. We have identified five amino acids that are involved in subunit communication at the dimer interface: R186, T192, S190, T09 and His133. We created a library of Fno variants to test our hypothesis. The Fno variants, R186Q, R186K, R186I, T192V, T192A, H133A, H133N and S190A were characterized using binding, steady-state and pre steady-state kinetic analysis and are discussed here.

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Abstract 2160

Human Cdc14 Phosphatase Stimulation by a Pseudosubstrate Motif

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Cdc14 is a widely conserved eukaryotic protein phosphatase from the dual-specificity subfamily of protein tyrosine phosphatases and regulates diverse cellular processes such as mitotic exit, cytokinesis, and DNA repair in different species. Cdc14 has been linked to hearing loss and male infertility in humans and mice, while in multiple fungal species Cdc14 has been linked to pathogenesis. Our lab is interested in understanding the catalytic mechanism, regulation, and biological functions of Cdc14 enzymes. Recently, we discovered a pseudosubstrate motif in all fungal Cdc14 enzymes that plays an important role in the catalytic cycle by binding the active site to stimulate the rate-limiting phosphoenzyme hydrolysis step, and provides a mechanism for cells to regulate Cdc14 activity. While this pseudosubstrate motif is not conserved in animal Cdc14 enzymes, AlphaFold predictions of human Cdc14 structures reveal a different motif interacting with the active site that is well conserved in metazoan Cdc14 orthologs and could perform a similar function. The goal of my project was to test if this motif is mechanistically similar to the fungal pseudosubstrate motif. To accomplish this, I created mutations in residues predicted to bind in the Cdc14 active site based on the AlphaFold structure and tested their effect on enzyme activity compared to wild-type Cdc14 using steady-state kinetic assays. I observed a reduction in both the kcat and KM, very similar to the effects observed with mutations in the fungal motif, and most consistent with a contribution to the rate-limiting catalytic step. These data confirm the predicted pseudosubstrate motif in human Cdc14 acts in the same way as the previously characterized fungal motif and implies that all Cdc14 enzymes use a pseudosubstrate motif in their disordered C-terminus to stimulate and regulate activity. Future work will focus on understanding the biological significance of the pseudosubstrate motif in Cdc14 enzymes.

Purdue University Department of Biochemistry, Purdue University College of Agriculture AgSeed Program.

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Abstract 2174

The surprising connection between lanthanides and methylotrophy

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The recent discovery that metals known as lanthanides, have an active role in metabolism, particularly in methylotrophy, has opened avenues to resolving long-standing metabolic mysteries across diverse biological systems. Until recently, it was believed that methanol oxidation was catalyzed in the periplasm solely by the extensively studied Ca- and pyrroloquinoline quinone (PQQ)-dependent methanol dehydrogenase (MeDH) encoded by the mxaFI genes (MxaFI). It is now recognized that methanol oxidation can be driven by the periplasmic MeDH XoxF, which utilizes lanthanide cofactors in place of Ca. XoxF is highly divergent but widespread in environmental strains including all methylotrophs and numerous non-methylotrophs. My research program is leading efforts in defining the role of lanthanides in biology, focusing on the methylotrophic organism *Methylobacterium extorquens* AM1. We have recently demonstrated far-reaching impacts of lanthanide biochemistry beyond methylotrophy: from the identification of new enzymes and pathways dependent on lanthanide chemistry to identifying the effects of lanthanide-dependent metabolism on the local environment and developing biological platforms for efficient recovery. Genetic studies confirmed that lanthanide metals are scavenged by using a parallel system to the well-studied, TonB-ABC-transport-dependent Fe scavenging pathway. The localization of the lanthanide during trafficking in the cell was possible by combining genetic and phenotypic studies with transmission electron microscopy. Two intriguing findings resulted from the integration of these studies: lanthanides are not readily bioavailable in the periplasm, and lanthanides are stored in the cytoplasm in mineral form. Secretion of a chelator for lanthanides (a.k.a. a lanthanophore) was identified when using poorly soluble forms of lanthanides. Transcriptomic analysis comparing the growth of strain AM1 with soluble vs poorly soluble lanthanide sources enabled the identification of the lanthanophore biosynthetic pathway. We are currently characterizing these lanthanide scavenging molecules, and additional peptides involved in REE trafficking. We have shown that engineered variants of *M. extorquens* cannot only use light lanthanides but also heavy lanthanides, enabling a microbial platform that can use numerous lanthanide sources including mining ores, electronic waste, and contaminated water streams. Finally, we assess how lanthanide use correlates with actinide use.

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Abstract 2182

A critical role for a CoxG homologue in the function of *Bacillus niacini* nicotinate dehydrogenase

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N-Heterocyclic aromatic compounds (NHACs) are a group of compounds prevalent in manufactured products, such as pesticides, pharmaceuticals, and dyes. As such, NHAC contamination of soil and ground water is a widespread concern. Several common soil bacteria are capable of transforming NHACs into useful metabolites, including *Bacillus niacini*, *Pseudomonas putida* and *Eubacterium barkeri*. The mechanisms by which these bacteria are capable of NHAC catabolism is of interest for bioremediation strategies. Nicotinic acid (NA) has been used as a model system for determining the mechanisms of NHAC degradation. The pathways for NA degradation in *P. putida* and *E. barkeri* have been elucidated, but a relatively large portion of the analogous pathway in *B. niacini* remains uncharacterized. Previous work has identified 5-genes within a larger cluster that is upregulated in *B. niacini* when cultured in the presence of NA. A Pseudomonas expression vector (pBBr) containing these 5 genes was used to transform a non-NHAC degrading *Pseudomonas fluorescens* 1f2 cell line. In-cell assays were performed with transformants incubated in minimal media with NA. HPLC analysis of media samples over time showed evidence of degradation of NA, formation and degradation of 6-hydroxynicotinic acid (6-HNA), and formation of 2,6-dihydroxynicotinic acid (2,6-DHNA) – the first hypothesized intermediates within the NA degradation pathway. Based on these results, we hypothesize that the proteins encoded by this gene cluster, NicA1, NicA2, NicB1, NicB2, and CoxG, are involved in the first two steps of NA degradation in *B. niacini*, forming two distinct multimeric complexes with nicotinate dehydrogenase (NDH) and 6-hydroxynicotinate dehydrogenase (6-HNDH) activity. Using site-directed mutagenesis, single and double deletions of genes in the pBBr vector have been strategically performed to elucidate the role of each subunit for NDH and 6-HNDH activity. Activity of these mutants has been analyzed through in-cell assays with NA and 6-HNA, as above, using HPLC to track degradation over time. Current results indicate that all 5 genes are critical for both NDH and 6-HNDH activities. Previous attempts to reconstitute functional NDH and 6-HNDH complexes from *E. coli* have been unsuccessful due to the molybdenum ion, coordinated at the B subunits by the MCD cofactor, being absent. Recent results using an *E. coli* TP1000 strain with limited IPTG during induced expression indicate the presence of MCD, and possibly Mo2+, enabling functional analyses of the purified enzyme complexes with CoxG *in vitro*.

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Abstract 2186

Exploring Sortase Enzyme Intermediates and Conformational Dynamics with Molecular Simulations

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Sortase enzymes recognize a terminal sorting signal motif, consisting of 5 amino acids, to catalyze cell wall sorting reactions in gram-positive bacteria. These enzymes have use in the laboratory for sortase mediated ligation (SML) reactions. We present extensive atomistic molecular dynamics (MD) simulations of the transpeptidase Sortase B from *Bacillus anthracis*. Specifically, we explore the effectiveness of swapping the β7-β8 loop from different bacteria, *Staphylococcus aureus* and *Listeria monocytogenese*, for applications in protein engineering. MD simulations allow us to explore protein conformational dynamics of the different swap mutants, such as a tyrosine residue making interactions that disrupt the active site of the enzyme. Based on experimental kinetic data showing that the *Listeria monocytogenese* loop-swap has a higher catalytic activity than the wild type, but that the *Staphylococcus aureus* loop-swap has lost all activity, we designed a reliable set of order parameters or collected variables (CVs) to quantify differences in important fluctuations among the different systems. Observing important distances over the trajectory provides insight on how point mutations affect the protein dynamics. The Root-Mean Square Deviation (RMSD) of individual loop regions reveals the significance of loop swaps and possible connections between the ligand and the enzyme. A contact map and coordination number CV reveals affinities of the ligand to certain portions of the enzyme that lead to a deeper insight of the intermediate mechanism. This work highlights how biomolecular simulations can help to address questions arising from experimental observations of sortase swap mutants. Furthermore, atomistic simulations can help identify key interactions between the sortase substrate motif and contacts that are related to the change in enzyme activity of the enzyme. Using molecular docking, we can test the new connections by docking the target peptide motif and explore the binding poses with these new loop swaps.

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Abstract 2195

Identifying the NADH substrate binding site in 6-hydroxynicotinate 3-monooxygenase

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Bacterial degradation plays a significant role in the remediation of soils contaminated with N-heterocyclic aromatic compounds. These degradation pathways commonly involve ring hydroxylation and oxidative ring-opening reactions that convert them into common metabolites. Nicotinic acid catabolism by aerobic bacteria (e.g. *Pseudomonas putida*) serves as a model for exploring these mechanisms. Specifically, 6-hydroxynicotinate 3-monooxygenase (NicC) catalyzes the hydroxylation and decarboxylation of 6-HNA to 2,5-dihydroxypyridine. This reaction utilizes NADH as a substrate to reduce the FAD cofactor prior to forming oxygen-adducts necessary for catalyzing substrate hydroxylation. Although prior structural studies of NicC have indicated the FAD binding site, and mutagenesis studies have postulated the residues critical for 6-HNA binding, the binding site for NADH remains unknown. Structural analysis of NicC suggests the NADH may be bound at a unique site compared to other class A monooxygenases. This study aims to determine if K204, K300 and N352, residues in this unique site, are essential for NADH binding or NAD+ dissociation. The side chain of K204 is located near the isoalloxazine ring in close juxtaposition to the 6-HNA binding site and is hypothesized to play a role in cationic repulsion of NAD+ after flavin reduction. Side chains of K300 and N352 are both located near the entry of the putative NADH site. The location of these residues supports potential binding interactions with the phosphate backbone, ribose ring, and adenine ring. The K204A, K204M, K300M and N352L mutations have been generated by site-directed mutagenesis. Purified K204A variant of NicC does not bind an appreciable amount of FAD, prohibiting functional analysis. The purified K300M variant displays sufficient FAD binding to enable kinetic analysis. In comparison to the WT NicC enzyme, the K300M NicC variant displays a 10-fold higher KM for NADH and a 2-fold slower kcat, suggesting that K300 may play a role in NADH binding, consistent with the hypothesis. Comparisons of the observed changes in KM for NADH and kcat for the K204M and N352L variants will be presented, as well as the effects of each variant on the rates of anaerobic reduction of enzyme-bound flavin with varying concentrations of NADH by stopped-flow spectrophotometry.

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Abstract 2199

Effects of intracellular conditions on the pyruvate substrate inhibition of Lactobacillus casei L-lactate dehydrogenase

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Although up to 25% of all enzymes show substrate inhibition, this kinetic mechanism often disregarded as an artifact of *in vitro* reaction conditions. This assumption is often made, however, without consideration or investigation of factors that may enhance or diminish substrate inhibition. Here, the kinetics of *Lactobacillus casei* L-lactate dehydrogenase (Lc-LDH) are investigated under a variety of conditions to better understand whether substrate inhibition persists using reaction conditions that more closely mirror those of a bacterial cell. When reaction conditions simulate the ionic strength inside a cell, the half-saturating concentration of the activator fructose 1,6-bisphospahte (FBP) shows a 21-fold increase. However, at saturating FBP concentrations, pyruvate substrate inhibition was found to be insensitive to changes in ionic strength. In contrast, when the highly crowded intracellular milieu was mimicked through the addition of Ficoll 70, the pyruvate-dependent kinetics were significantly altered. Increasing concentrations of Ficoll resulted in an increase in pyruvate Ki with a simultaneous decrease in Km. Similar changes in pyruvate Km and Ki were also observed as the concentration of FBP was increased from micromolar to millimolar concentrations. Since increasing concentrations of FBP have previously been shown to favor the R-state of Lc-LDH, the similar effects caused by the addition of FBP and Ficoll suggest that both alter the inherent T/R equilibrium present in solution. To further probe a potential shift in the conformational ensemble, Lc-LDH was assayed in the presence of ATP, as recent literature has suggested that ATP confers a stabilizing effect on specific protein conformations. Addition of ATP at concentrations comparable to those found in bacterial cells resulted in complete abolishment of substrate inhibition for Lc-LDH. Overall, these results point to a dynamic equilibrium of Lc-LDH conformations that is highly sensitive to assay conditions. Further, as reactions conditions more closely approximate those of a bacterial cytoplasm, it appears that enzyme conformations that are insensitive to substrate inhibition become favored. Our results suggest that although substrate inhibition is observed *in vitro* for Lc-LDH, it may not be operative *in vivo*. This contrasts with the literature surrounding mammalian lactate dehydrogenases speculating that substrate inhibition is still operative *in vivo*. Our findings point to an additional unexplored difference in LDH regulation between mammalian and bacterial systems.

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Abstract 2200

The impact of patient mutations on coenzyme B12 loading and repair

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Adenosylcobalamin (AdoCbl) or coenzyme B12 serves as cofactor for methylmalonyl CoA mutase (MCM), which converts methylmalonyl-CoA to succinyl-CoA and serves an anaplerotic function. The MCM reaction is initiated by homolytic cleavage of the cobalt-carbon bond, generating a reactive 5′-deoxyadenosyl radical, which is occasionally lost, leaving inactive cob(II)alamin in the active site. Inactive MCM is repaired by the chaperones, adenosyltransferase (ATR) and CblA, a GTPase. Mutations in MCM, ATR or CblA lead to methylmalonic aciduria, an inborn error of metabolism. We have recently obtained the crystal structure of the human MCM-CblA complex in the presence of GDP and CoA. The structure reveals a large movement of the B12 domain of MCM and ordering of highly mobile regions in CblA. The interface is formed by contacts between CblA and both the substrate and the B12-binding domains of MCM. Herein, we present a biochemical analyses of patient mutations located at the MCM-CblA interface, providing a rationale for their pathogenicity. MCM enhances the GTPase activity of CblA 55-fold and serves as a GTPase activating protein (GAP). While mutations in the substrate domain of MCM (R154H, D156N and R228Q) show modest effects, mutations in the B12 domain (R616C and R694W) severely impair GAP activity. Mutations in CblA (R98G and R209S) located at the MCM-CblA interface have no effect on the intrinsic GTPase activity, but GAP activation by MCM is almost completely abolished. Additionally, AdoCbl loading onto MCM as well as cob(II)alamin off-loading are impaired in mutants with low GAP activity.

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Abstract 2208

Characterizing the structural and biochemical consequences of IMPDH2 mutation

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Cells widely utilize purine nucleotides and nucleosides as signaling molecules, energy sources, and metabolites. Purine nucleotides can be synthesized either through salvage pathways or de novo pathways. In most organisms, the enzyme inosine 5′-monophosphate dehydrogenase (IMPDH) catalyzes the rate-limiting step in the de novo biosynthesis of guanine nucleotides and controls the metabolic branch point between adenine and guanine nucleotide biosynthesis. Allosteric regulation of human IMPDH involves its assembly into filaments, which are affected by the binding of adenine and guanine nucleotides to the regulatory domain of the enzyme—ATP binding induces formation of filaments which are catalytically active, while GTP binding induces compression of these filaments, inhibiting catalysis. There are two isoforms of IMPDH in humans—IMPDH1 is constitutively expressed at low levels, and IMPDH2 expression is upregulated in proliferating cells. Point mutations around the regulatory domain of IMPDH2 have recently been linked to neurodevelopmental disorders. Using negative stain electron microscopy, cryo-electron microscopy, and biochemical assays, we are defining the effects of each mutation on filament structure and enzyme activity. Here, we show that each mutation effectively desensitizes the enzyme to GTP inhibition. While all IMPDH2 mutations result in this similar biochemical defect, the structural consequences of the mutations vary. For one mutation, L245P, we present cryo-EM structures that show the mutation shifts the conformational equilibrium towards the active conformation, disfavoring compression of the filament. Our biochemical and structural characterization of each variant provides insight into the mechanisms of IMPDH2-associated neurodevelopmental disorders and reveals gaps in our understanding of IMPDH2 allosteric regulation.

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Abstract 2214

Evidence of a Catalytic Dyad in F420-Dependent Glucose-6-Phosphate Dehydrogenase Using Steady-State and Pre Steady-State Kinetic Methods

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Lindsay Davis, Ana Alvarez, Kayunta Johnson-Winters, Ghader Bashiri, Edward Baker

F420-dependent glucose-6-phosphate dehydrogenase (FGD), although initially discovered in Nocardia, has been extensively studied in Mycobacteria due to health relevance for extreme drug resistant forms of tuberculosis disease (TB). FGD catalyzes the conversion of glucose-6-phosphate (G6P) to 6-phosphogluconolactone, similar to the first step in the pentose phosphate pathway. Our research has focused on elucidating the catalytic mechanism of this enzyme. Previous pH profiles revealed that Glu109 served as the active site acid, while the functionality of His40, previously proposed to be the active site base, still remains unknown. Here, we have continued our investigation of His40, while studying the role of the active site residue, Glu13 in search of which amino acid serves as the active site base. Using site-directed mutagenesis, we created several FGD variants of the beforementioned residues including Glu13Ala, Glu13Gln, His40Ala, and His40Gln. These variants were kinetically characterized using binding experiments, steady-state and pre steady-state kinetic methods, in addition to PROPKA calculations and pH dependence studies. The binding studies suggest that these conserved amino acids are important for the binding of F420 but are not involved in G6P binding. The pH profiles along with the PROPKA calculations suggest that Glu 13 and His40 function as a catalytic dyad, with His40 donating a proton to Glu 13. His 40 can then act as a base, abstracting a proton from G6P facilitation the reduction of the F420 cofactor. The pre steady-state kinetics studies suggest that hydride transfer is not rate-limiting in catalysis, while the global fitting of wtFGD reveal that the reaction follows a fast kinetic/slow product release mechanism with no observable intermediates. Future work will focus on fine-tuning the global analysis of wtFGD along with the FGD variants using Kintek Explorer to fully understand the role of each amino acid during catalysis.

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Abstract 2246

Mechanism-based Inhibitors and Site-Specific Selenocysteine Substitution as Tools for Understanding Enzymatic Thiyl Radical Chemistry

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Cysteine-localized thiyl radicals are versatile cofactors in biochemistry and are essential for nucleotide metabolism, anaerobic glycolysis, and enantioselective radical rearrangements. The utility of thiyl radicals in diverse chemical transformations is due to their reactivity as labile H-atom donors and acceptors or as radical nucleophiles. This privledged reactivity, as well as the nature of their characteristic optical and paramagnetic fingerprints has significantly challenged experimental investigation of thiyl radical chemistry, limiting our knowledge of the mechanisms by which thiyl radicals activate substrates and are manages within the enzyme active site, which may contain endogenous weak C-H bonds. We have developed a suite of methodologies for investigating the mechanism of thiyl radicals in catalysis, including mechanism-based inhibitors, chemical radical traps, and site-specific substitution of cysteine for selenocysteine. Selenocysteinyl radicals exhibit approximately a 500 mV depression of the radical reduction potential relative to cysteine thiyl radicals, allowing us to alter the thermodynamic landscape of radical transfer and catalysis. Using these methods we demonstrate the enantioselective substrate radical quenching by C199 in the enzyme NikJ from Streptomyces tendae, involved in the biosynthesis of nikkomycins. We further employed various methacrylate isotopologs and selenocysteine substitution to evidence two thiyl radicals in the mechanism of pyruvate formate lyase. We anticipate these methodological tools to be of broad interest in the study of thiyl radical enzymes and will reveal new insight into their mechanisms and how they may be targeted therapeutically.

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Abstract 2252

Investigation of digestive enzyme inhibition by *Ginkgo biloba* leaf extracts

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Ginkgo biloba, a popular, hardy tree with fan-shaped leaves, has survived largely unchanged since the time of the dinosaurs. Ginkgo leaf extracts are a widely consumed dietary supplement believed by users to improve blood flow and slow the aging process. Although the leaves are rich in flavonoids and other anti-oxidant compounds, controlled experiments have not been able to confirm medicinal benefits. It is known, however, that the supplements frequently cause gastrointestinal difficulties including upset stomach, nausea, bloating, and diarrhea. To determine the possible source of the gastrointestinal problems, we analyzed the effect of Ginkgo leaf extracts on a series of digestive enzymes responsible for breaking down various components of the diet, including carbohydrates (amylase), proteins (chymotrypsin), and fats (lipase). The ability of green and senescent Ginkgo leaf extracts to inhibit digestive enzymes was tested under simulated physiological conditions and compared to known enzyme inhibitors. Commercial supplements were compared to extracts prepared in our laboratory from leaves collected locally. We also investigated whether or not the inhibitory properties could be mitigated by additives. This research is supported by the Office of Naval Research Midshipman Research Fund and the United States Naval Academy Chemistry Department.

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Abstract 2270

Cobalt-sulfur coordination chemistry drives B12 loading onto methionine synthase

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Vitamin B12 is sequestered, tailored, and delivered via an elaborate trafficking pathway from its point of entry into the cell to the two known enzymes that utilize it: cytoplasmic methionine synthase (MS), and mitochondrial methymalonyl-CoA mutase. Clinical genetics studies on patients with inborn errors of cobalamin metabolism have led to the identification of at least seven genes involved in the B12 trafficking pathway. Mutations in the cytoplasmic methylcobalamin (MeCbl) branch lead to homocystinuria, while mutations in the mitochondrial 5′-deoxyadenosylcobalamin (AdoCbl) branch lead to isolated methylmalonic aciduria. Studies on the mitochondrial chaperones have provided detailed mechanistic and structural understanding of the AdoCbl branch of the trafficking pathway. In contrast, little is known about the MeCbl branch, how vitamin B12 is delivered to MS, and the roles of two chaperones, MMACHC and MMADHC (or CblD), in this process. Our laboratory recently discovered that CblD binds B12 via an unusual cobalt-sulfur bond, which is rare in Nature. The existence of this rare coordination chemistry was confirmed by an X-ray crystal structure of the CblD-B12 complex, which also suggested how B12 might be transferred from this chaperone to MS. Herein, we report that a sulfur ligated cobalamin species on CblD can transfer B12 to methionine synthase. Biochemical and mutation analysis of key residues support a mechanism for sulfur chemistry-based transfer of B12 from CblD to MS in an inter-protein complex. In summary, this study has been instrumental for assigning a function to CblD, which had been previously elusive. In addition, we have discovered how novel coordination chemistry is used to translocate the cofactor cargo to the cytoplasmic target, MS.

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Abstract 2274

Effect of pH on the pyrroline-5-carboxylate (P5C) reductase activity

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The gene *proC* (SMc02677) from the nitrogen fixing symbiotic bacteria *Sinorhizobium melloti*, is predicted to encode for a Δ^1-pyrroline-5-carboxylate (P5C) reductase, an enzyme that catalyzes the ultimate step in proline biosynthesis, the NAD(P)H-dependent reduction of P5C to L-proline. Previous work in our laboratory demonstrated that L-proline was not a viable substrate for P5C reductase monitoring the reverse reaction, the $NAD(P)^+$ dependent oxidation of L-proline. However, the L-proline analog L-4-thiazolidinecarboxylate was readily oxidized under the same conditions in the presence of $NAD(P)^+$. To better understand the role that the enzyme plays in catalysis, we investigated the dependence of k_{cat} and K_m on the pH of the solution by measuring the kinetic parameters at 0.5 pH increments. Based on a proposed mechanism, we predict that the kcat would increase with increasing pH because a protonated ring nitrogen on the substrate at low pH would have an electron withdrawing effect making the hydride transfer less favorable. Whereas the ring nitrogen would be deprotonated at high pH and the electron donating effect of the lone pair would make hydride transfer more favorable. It is possible that the protonation state of the active site and/or the substrate could also lead to changes in Km, but the effect is hard to predict *a priori*. The kinetic activity of P5C reductase was monitored using UV-visible spectrophotometry following the reduction of $NAD(P)^+$ at 340 nm using the L-4-thiazolidinecarboxylate as the substrate at pH increments ranging from 6.0 to 10.0. The catalytic efficiency (k_{cat}/K_m) and kinetic parameters of the enzyme were compared. Preliminary analysis showed the k_{cat} increased as pH increased whereas the K_m appeared to be relatively unaffected. These results are consistent with the hypothesis that the deprotonated ring nitrogen at higher pH enhances the rate of hydride transfer.

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Abstract 2287

Exploring the potential binding multiplicity of NADH in 6-hydroxynicotinate 3-monooxygenase

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6-Hydroxynicotinate 3-monooxygenase (NicC) is a class A flavin-dependent enzyme that catalyzes the decarboxylative hydroxylation of 6-hydroxynicotinic acid (6-HNA) to 2,5-dihydroxypyridine (2,5-DHP) with concomitant oxidation of NADH. Previous modeling of kinetic studies suggests the possibility that two NADH molecules are bound during catalysis. Structural analysis of the FAD-bound enzyme indicates two possible binding sites for NADH with both sites within NicC having similar predicted electrostatic properties. Intriguingly, although one of these sites in NicC is homologous to the NADH binding site observed in para-hydroxybenzoic acid hydroxylase, the quintessential class A flavin monooxygenase used to model this enzyme class, the second NADH binding site appears to be relatively unique to NicC. Thus, we are interested to determine whether both sites in NicC bind NADH. First, we observed that product NAD+ shows mixed inhibition with respect to NADH, further supporting the possibility of two functional NADH binding sites. Next, kinetic analysis of the effect of changing the His211 residue in the putative unique NADH binding site to alanine showed that the rate constant for anaerobic (single-turnover) FAD reduction by NADH dropped from 44 s-1 (WT) to 14 s-1 (H211A), providing additional functional evidence to support a role for NADH binding at this unique site. To further elucidate which potential NADH site, or both, plays a functional role in NicC catalysis, variants of the enzyme (K300A in the unique site, W273A and H162A in the "conserved" site) were generated by site-directed mutagenesis and purified by affinity chromatography for further comparative kinetic analyses.

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Abstract 2314

Kinetic Characterization of the Threonine 124 Alanine Variant of Pyrroline-5-Carboxylate Reductase from *Sinorhizobium melloti*

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Nathan Bruender

The advancements in high-throughput genomic sequencing that decreased time and increased ease of full genome sequencing have led to many gene and subsequently predicted protein sequences deposited in databases. However, the functional characterization of the putative gene products has significantly lagged behind the deposition of sequences creating a bottleneck in structure-function studies. One example that was explored in our lab was the gene proC (SMc02677) from the nitrogen fixing symbiotic bacteria *Sinorhizobium melloti*, which was identified to encode for a putative Δ^1-pyrroline-5-carboxylate (P5C) reductase. Homologs of the enzyme catalyze the NAD(P)H-dependent reduction of P5C to L-proline, which is the final step in L-proline biosynthesis in both prokaryotic and eukaryotic organisms. Previous work in our laboratory demonstrated that L-proline was not a viable substrate for P5C reductase monitoring the reverse reaction, the NAD(P)+ dependent oxidation of L-proline. However, the L-proline analog L-4-thiazolidinecarboxylate was readily oxidized under the same conditions in the presence of NAD(P)+. Using the substate analog, we wanted to explore the catalytic mechanism of the enzyme, specifically exploring the role that Threonine 124 (T124) may play in catalysis and/or binding. Performing multiple sequence alignments across evolutionarily diverse sources of P5C reductase shows that T124 is strictly conserved, and structural analysis of homologs shows the conserved residue is in the active site near the NAD(P)+ and L-proline (L-4-thiazolidinecarboxylate) binding site suggesting it may have an important role in the enzyme's function. A variant was produced via site-directed mutagenesis where Threonine 124 was replaced with Alanine (T124A), the gene was recombinantly expressed in *Escherichia coli*, and the recombinant protein was purified. The kinetic activity of the T124A variant was monitored monitoring using UV-visible spectrophotometry following the reduction of NAD(P)+ at 340 nm using the L-4-thiazolidinecarboxylate as the substrate. The kinetic parameters of the T124A were compared to the wild-type activity. Preliminary analysis shows that the catalytic efficiency was 18-fold less than wild type. These results suggest that T124 plays a role in catalysis, however, due to the modest decrease in efficiency, it does not appear to be essential in the catalytic mechanism.

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Abstract 2356

Malate dehydrogenase-citrate synthase multi-enzyme complex formation is associated with the pH changes in the mitochondrial matrix in living yeast cells

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The malate dehydrogenase (MDH)-citrate synthase (CS) multi-enzyme complex is an evolutionarily conserved metabolon enhancing the forward reaction of the Krebs tricarboxylic acid (TCA) cycle through substrate channeling. Although the dynamic formation of the MDH-CS metabolon has long been postulated as a regulatory mechanism of the TCA cycle flux, the relationship between the MDH-CS complex formation and cellular metabolic status, and the mechanisms regulating its association has not been experimentally proven. In the previous meetings, we have reported that MDH-CS interaction depends on the presence of fermentative and non-fermentative respiratory substrates in living *Saccharomyces cerevisiae* cells, suggesting the dynamic regulation of the multi-enzyme complex formation in relation to respiratory fluxes. We have also reported that the MDH-CS interaction is affected by TCA cycle intermediates, NAD(H), and ATP availability, and pH *in vitro*. Therefore, we hypothesized that the changes in the physicochemical conditions in the mitochondrial matrix under different respiratory conditions affect the interaction of the MDH-CS complex and impact the TCA cycle flux. To monitor the relationship between the MDH-CS interaction and redox state, ATP abundance, and pH, we expressed roGFP2, mitGO-ATeam2, and pHluorin fluorescence sensors, respectively, in the mitochondrial matrix of the yeast cells with the genomic insertion of nonoBIT split-luciferase modules to detect the MDH-CS interaction. When the MDH-CS interaction was disrupted in association with the respiratory shift from oxidative phosphorylation to fermentation by the addition of glucose, the pH in the mitochondrial matrix slightly increased. Based on our *in vitro* results, increase in pH reduced the MDH-CS complex affinity. Contrarily, ATP content and NADH redox state did not show any relationship with MDH-CS complex dissociation induced by the addition of glucose. When the raffinose-grown yeast cells were treated with various respiratory inhibitors, mitochondrial matrix pH was altered. Inhibition of respiratory complex III and IV by antimycin A and sodium cyanide, respectively, led to a decrease in mitochondrial matrix pH which concerted with the increase of MDH-CS association. However, the mitochondrial matrix pH was not affected by malonate and oligomycin, although these inhibitors significantly affected the MDH-CS interaction. Considering our *in vitro* results showing the reduced affinity of the MDH-CS complex in high pH, these results support our hypothesis and suggest that mitochondrial matrix pH is a novel factor involved in regulating the TCA cycle metabolon dynamics. However, pH will not be the sole factor regulating metabolon formation, and other unknown factors

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may play a dominant role in certain conditions, such as in the presence of specific respiratory inhibitors.

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Abstract 2379

Regulation of the *Saccharomyces cerevisiae* PAH1-encoded phosphatidate phosphatase by citric acid cycle metabolites

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Phosphatidate phosphatase (PAP) catalyzes the conversion of phosphatidate to diacylglycerol in a reaction that depends on Mg2+. PAP enzymes are evolutionarily conserved from yeast to mammals. In the model yeast *Saccharomyces cerevisiae*, the *PAH1*-encoded PAP regulates lipid biosynthesis by controlling the levels of phosphatidate and diacylglycerol, which act both as lipid precursors and signaling molecules. The enzyme activity of the Pah1 PAP is regulated by phospholipids, sphingoid bases, and nucleotides. In this work, we examined the regulation of the PAP activity of Pah1 by citric acid cycle metabolites (e.g., citrate, isocitrate, and α-ketoglutarate). A unit of PAP activity was measured by following the release of 32P-phosphate from the radiolabeled substrate 32P-phosphatidic acid. The results showed that the PAP activity of Pah1 increased by 2-fold, 1.8-fold, and 1.6-fold in the presence of citrate (1 mM), isocitrate (1 mM), and α-ketoglutarate (2.5 mM), respectively. Next, we examined the effects of citrate and isocitrate on the kinetics of Pah1 PAP activity with respect to the concentration of Mg+2 ions. The results showed that the highest activity was obtained in the presence of 1 mM of Mg+2 ions and 1 mM organic acids. Currently, we are examining the effects of citrate and isocitrate on the kinetics of Pah1 activity with respect to the surface concentration of PA. These results corroborate our previous findings with the PAP activity from the yeast *Yarrowia lipolytica*, which is also regulated by citrate metabolites. These findings indicate that the effect of citrate metabolites on PAP enzymes could be widespread among yeasts and, perhaps, mammalian enzymes.

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Abstract 2399

Isolation and Purification of a Photoreactive Flavoprotein from Schmidtea mediterranea

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Photolyase (PL) and Cryptochromes (CRY) share highly conserved sequences, performing different functions as photoreactive flavoproteins. Cryptochromes regulate circadian rhythm in light entrainment mechanisms and photolyases are responsible for the correction of cyclobutane thymine dimers. These dimers are produced when DNA is damaged by UV light. These lesions are hazardous, and without correction result in carcinogenic effects or cell death. Schmidtea medditeranea, otherwise known as planaria, is a photophobic freshwater flatworm that contains a PL/CRY and the corresponding gene has not been previously characterized. Here we present the results from our attempts to develop a simple expression system for one step downstream protein purification using a polyhistidine tag. The protein has been isolated with a molecular weight of ~62 kDa. We have also presented here preliminary spectroscopic data on the successful cloning and characterization of the composition of the protein.

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Abstract 2417

Phosphorylation of human mitochondrial MDH2: Impact of enzyme structure and function

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Metabolon formation of Malate Dehydrogenase (human mitochondrial MDH: hMDH2) and Citrate Synthase (CS) drives the thermodynamic coupling and efficient substrate shuttling within the tricarboxylic acid cycle. However, the formation of this complex is regulated allosterically by its substrates and other metabolites. Depending on the cell's energy state and metabolic needs, the MDH-CS interaction may need to be shifted to support other metabolic pathways. There needs to be a current understanding of how this metabolon forms. This study investigated the phosphorylation of MDH as a possible mechanism for describing the changes in the structure and function of MDH that may regulate enzymatic activity. Phosphomimic hMDH2 mutants (S45D, T85D, S222D, and T224D) were generated to investigate how phosphorylation may regulate enzymatic activity. His tagged recombinant hMDH2 proteins were purified, and the specific activity, Km, and Vmax were determined and compared to each Phosphomimic. hMDH2S45D showed a significant reduction in specific activity by 43% compared to wildtype. hMDH2S222D specific activity was almost below detection. Yet, two mutants, T85D and T224D were two and six-fold greater than wildtype hMDH2, respectively. Such changes in hMDH2 activity indicate phosphorylation's diverse and essential unknown effect on MDH function. We then measured the impact on the melting point of each MDH alone and with the allosteric metabolite regulators. Each Phosphomimic displayed a distinct melting pattern or shift from the wildtype stability. We next used Alphafold to predict how the protein phosphorylations would impact the tertiary structure of the hMDH2. The program H++ was used to predict how the Phosphomimic installation changed the protein's local environment. With this evidence, a search of possible kinases was conducted using online databases, which were then selected based on known phosphorylation sites and predicted data. Lastly, a kinase assay was conducted using kinases predicted with a likelihood above a certain threshold to determine the activity of each kinase to phosphorylate MDH. Overall this study helps identify a mechanism of regulation for this crucial metabolic enzyme.

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Abstract 2418

Potential Impact of Phosphorylation of Cytosolic Malate Dehydrogenase

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Human Malate Dehydrogenase (hMDH2) is an enzyme which catalyzes the reversible reaction of oxaloacetate (OAA) to malate. This study focuses on human cytosolic MDH (hMDH2) from humans which is important in the regulation of various biochemical pathways such as the aspartate malate shuttle, gluconeogenesis, and adipogenesis. Previous literature suggests that there is more MDH activity in cancerous cells (lung tumors) than normal cells. This study attempts to understand if phosphorylation can regulate hMDH2 and give a deeper understanding to its potential impact in cancerous MDH. Through a meta-analysis of several phosphorylation databases, we have found 22 experimentally identified phosphorylation sites. No previous research has been done to understand the impact of phosphorylating such sites and how that can impact hMDH2's interactions with other proteins. We believe that phosphorylation of certain sites on cytosolic canonical MDH as well as its splice variant 3 can change the configuration of MDH which can reveal new properties of the enzyme in terms of its structure and function. Such changes can change the affinity of MDH to various proteins which in turn can potentially stop or help certain pathways that cytosolic MDH is a part of. In order to test this hypothesis, we will use phosphomimics to imitiate phosphorylation by changing potential serines (S) into aspartic acids (D). From the 22 sites, three were chosen to test:(S108D, S236D, and S130D). These sites were chosen based on their potential interactions with four proteins: Glutamic Oxaloacetic Transaminase (GOT1), phosphoenolpyruvate carboxykinase (PEPCK), Malic Enzyme, and ATP Citrate Lyase. S108D was chosen because it is a potential site where all 4 proteins bind. S236D was chosen because it is a potential interface region. S130D was chosen because it is a potential binding site to GOT1. Through the measurements of Kmv, specific activities, and thermal melts we can find if hMDH2 structure and function has changed. If phosphorylation can potentially impact pathways of hMDH2 and its interaction with proteins, then MDH can be targeted within the tumor cells and can regulate its pathways in a way so the tumor cannot grow. Phosphorylation and its impact on MDH can be a potential approach to treat fast growing tumor cells..

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Abstract 2425

Possible role of phosphorylation of human cytosolic MDH on its structure and function

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Olivia Heras

Cytosolic malate dehydrogenase (human: hMDH1) catalyzes the redox reaction of malate and oxaloacetate (OAA). While the thermodynamically favorable reaction is in the OAA-malate direction, there are several cytosolic metabolic pathways where hMDH1 integrates in either direction. Some metabolite pathways hMDH1 is involved in includes the aspartate-malate shuttle, gluconeogenesis, NADH-NADP hydride transfer, adipogenesis and glutamine metabolism. How MDH integrates into these pathways is unknown. One possible mechanism for the control of metabolic enzymes is through post-translational regulation and allosteric regulators. Phosphorylated can drive regulation through a change in structure and function. Using phosphorylation databases, an analysis was performed to determine 22 potential phosphorylation sites of the canonical and splice variant three of human cytosolic MDH. Cytosolic MDH shares oxaloacetate and malate with at least 4 other proteins including, ME1, PEPCK, GOT1, and ACL. There are several studies indicating possible protein-protein interactions between hMDH1 and these proteins. Analysis of putative hMDH1 binding protein was conducted and the interface regions aligned with the experimentally determined phosphorylation sites. The three amino acid residues were found to lie in several of the possible protein interface (hMDH1 S260, hMDH1 S42, and hMDH1 S328). To mimic the impact of phosphorylation we performed site directed mutagenesis from a Ser to Asp. The impact of the phosphomimic on kinetic function of each enzyme was determined. Changes in structure are determined by shifts in the melting temperature of each protein. Together we show an role of MDH in controlling a diverse set of metabolic pathways.

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Abstract 2432

Putative Regulation of Human Mitochondrial Malate Dehydrogenase Through Phosphorylation

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The last step in the tricarboxylic acid cycle involves the regeneration of Oxaloacetate (OAA) from malate catalyzed by malate dehydrogenase (human mitochondrial MDH; hMDH2). The oxidation of malate to OAA is thermodynamically possible as it is coupled to the following enzyme in the pathway citrate synthase (CS). While this interaction has been demonstrated for years, the interface and critical residues have yet to be clarified. Because mitochondrial MDH is also involved in other pathways independent of CS it is logical that such interactions are dynamically regulated. Recently, evidence of a dynamic complex between MDH and CS influenced by metabolites was demonstrated. This work supports our hypothesis that several unknown factors can regulate the interaction between MDH and CS and thus control the flux of several integrated metabolic pathways. Meta-analysis of several phosphorylation mass spec databases uncovered 38 known phosphorylation sites in mammalian MDH2, all of which have yet to be examined in the literature. Residues S8, T37, and Y56 were chosen for further study. Each residue is located in a cluster near the interfacing site on the two predicted docking models and is solvent-exposed, increasing possible access for a protein kinase. To better understand how phosphorylation of hMDH2, each residue, was mutated to Asp to mimic the chemistry of phosphorylation. Because in pyruvate kinase M2, phosphorylation changes the structure and enzyme's ability to be activated by metabolites, we hypothesize these residues play a similar role. As citrate, OAA, malate, a-ketoglutarate, and ATP increase the affinity of MDH with CS and are also possible allosteric regulators of MDH. We examined the impact of each metabolite on hMDH2 phosphomimetic. We also determined the melting point of each protein to identify structural changes caused by the phosphomimetic mutants. Kinetic analysis of each phosphomimetic was determined to understand the possible regulation by phosphorylation. Preliminary SAXS data on MDH, CS, and complexes suggest a configuration for the MDH-CS complex and show subtle changes due to small molecule additives. These findings point towards a previously unknown mechanism for the regulation of hMDH2.

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Abstract 2437

Snapshots from the catalytic landscape of chalcone isomerase

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Chalcone isomerase (CHI) is an enzyme in the flavonoid pathway of plants that catalyzes the cyclization of 3-ring flavonoid scaffolds. Existing models of CHI catalysis disagree upon on whether CHI binds exclusively to the cyclization-productive, s-trans conformer of the chalcone substrate, or if CHI is also binds the non-productive, s-cis conformer and then facilitates a conformational rearrangement from s-cis to s-trans in the active site. To help resolve this question, we first used a differential scanning fluorometry-based binding assay to identify a substrate analogue which binds well to CHI and is also capable of s-cis to s-trans isomerization-like rotations. The substrate analogue, phloretin, was selected and soaked into protein crystals of a type I-CHI from Medicago truncatula (MtCHI-I). The protein X-ray crystal structure reveals eight protein molecules in the asymmetric unit. The eight enzyme active sites each reveal different conformers of phloretin ranging from near cyclization-productive (s-trans) to non-productive (s-cis). When viewed together, these snapshots depict a series of coordinated, dynamic, chemical interactions that lower barriers to substrate rearrangements approaching stereoselective bond formation. Overall, this work supports a catalytic model for CHI in which the enzyme operates not as a static, pre-organized active site molded to a transition state, but rather as a dynamic, catalytic ensemble.

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Abstract 2449

Phosphate binding in PNP alters transition state analog affinity and subunit cooperation

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Purine nucleoside phosphorylases (PNP) are essential enzymes in the purine salvage pathway. Genetic deficiency of PNP leads to the accumulation of 2′-deoxyguanosine (2′-dGuo) in plasma and dGTP in activated T-cells to cause arrest in DNA synthesis and T-cell apoptosis. Immucillin-H (Mundesine), a transition state analog (TSA) of PNP, has been approved for the treatment of relapsed and refractory T-cell lymphoma in Japan. PNP is also a potential drug target for autoimmune disorders, parasitic infections such as malaria, and toxoplasmosis because protozoan parasites are purine auxotrophs and lack the ability to perform de novo purine synthesis. PNP catalyze phosphorolysis of 6-oxypurine nucleosides with a PO4 oxyanion nucleophile. The substrates occupy distinct pockets in the PNP active site. Evaluation of the PO4 sites by mutagenesis, cooperative binding studies, thermodynamic and structural analysis demonstrate that alterations in the PO4 binding site renders PNP inactive and significantly impacts subunit cooperativity and binding to transition state analog inhibitors. Cooperative interactions between the cationic transition state analog and the anionic PO4 nucleophile demonstrate the importance of reforming the transition state ensemble for optimal inhibition with transition state analogs.

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Abstract 2469

Under Pressure: The Evolution of Drug Resistance and Production in Penicillium Fungi

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Genes and organisms often evolve in response to a specific driving force, as new functions are fixed in response to selective pressures. The presence of self-resistance genes in antibiotic producers creates a conundrum- how can antibiotic resistance evolve in the absence of the antibiotic, and how can an antibiotic producer evolve in the absence of resistance? This project focuses on the selective pressure that mycophenolic acid (MPA), an inhibitor of inosine monophosphate dehydrogenase (IMPDH), applies to the Penicillium fungal IMPDHs, some of which are MPA producers. Many Penicillium species contain two copies, "A" and "B", of IMPDH whereas the rest of the Trichocomaceae family have only one copy. The "A" copies of both an MPA producer and a non-producer are typical fungal IMPDHs; active and sensitive to MPA. The B copy from the MPA producer is also active and showed a 1000-fold increase in resistance towards MPA. However, the non-producer B copy is essentially inactive. With significant evolutionary distance between known producers, as well as protein phylogenetic distance between producer B copies, this research seeks to answer the following questions: How has MPA resistance and production evolved, are all non-producer B copies inactive, and why do non-producers retain inactive B copies? After generating a phylogenetic tree of available fungal IMPDHs, ancestor IMPDHs in the B copy subclade were calculated, and a selected modern and ancestor producer and non-producer B copy IMPDHs were assayed for activity and MPA resistance. Modern MPA producer B copies were all active and resistant, while non-producer B copies had a range of activity, though all measurably active B copies were found to be equally resistant to MPA. Most interestingly, two different ancestors at branch nodes between MPA producers were both inactive. This implies the absence of MPA in these ancestors between the modern MPA producers. The B copies of the modern producers and their more immediate ancestors were all active and resistant to MPA. The multiple occurrences of inactive modern B copy IMPDHs implied uncharacterized function, as genetic drift would be expected to remove nonfunctional genes. *In vitro* experiments revealed inactive B IMPDHs could form heterotetramers with A IMPDHs. Not only did dual expression *in vivo* result in heterotetramers, but, fascinatingly, the resultant hybrid gains around a magnitude of resistance to MPA, suggesting a possible function for inactive modern and ancestor B copy IMPDHs. Preliminary data suggests this mode of hybridization resistance holds with the non-producer Penicillium species originally found to have an inactive B copy IMPDH. In conclusion, MPA applies selective pressure towards

maintaining a secondary B copy IMPDH for resistance towards MPA through either intrinsic means or complementary heterotetramerization with primary A copy IMPDH, and MPA production more than likely stopped at some point in the history of the cluster in Penicillium.

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Abstract 2479

Understanding the limitations of a plastic-converting iron oxidoreductase

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Rieske oxygenases catalyze diverse reactions, many of which are keenly important for green chemistry applications. These applications include the biological conversion of waste polymers into valuable chemicals. Focusing on a dioxygenase-reductase pair from a polyethylene terephthalate (PET) plastic-consuming bacterium, this study aims at identifying why this enzyme and others like it have such poor total turnover numbers, with the goal of addressing their shortcomings. We have used a series of catalytic, thermodynamic, and spectroscopic approaches, including temperature-dependent small angle x-ray scattering and differential scanning calorimetry, to correlate loss of specific structural features with overall loss of function. We conclude that the quaternary structure of the dioxygenase is surprisingly stable, but weaknesses at the tertiary level limit both reductase and dioxygenase. This work paves the way for engineered improvements of both enzymes.

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Abstract 2491

Critical roles of redox carriers and the enzymes of carbon dioxide reduction

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Sheila Bonitatibus, Andrew Weitz

CO2 reduction processes in biology are widespread, often operating reversibility, and with minimal or zero over-potential. One such example are the enzymes of the OFOR superfamily (oxo-acid:ferredoxin oxidoreductase) which interconvert an oxo-acid, coenzyme-A and an iron-sulfur cluster bearing redox partner (a ferredoxin, or Fd), into CO2, a coenzyme-A adduct, and reducing equivalents in the form of reduced Fd. OFORs themselves are iron-sulfur cluster bearing enzymes that contain a thiamin pyrophosphate (TPP) moiety at the active site of the enzyme. The factors that govern the extent to which 'bias' can be achieved – how an enzyme might preferentially achieve CO2 reduction versus the oxidative chemistry – have yet to be understood at the level of mechanism and structure. Here, we have taken a multi-pronged approach, comparing OFORs that come from different organisms, with different physiologies. In our hunt for clues as what might compel an OFOR to engage in CO2 reduction preferentially, we have learned that the nature of the Fd redox partner may be as important as any other aspect of enzyme structure, in controlling bias. A combined enzymological, structural biology, spectroscopic, and protein electrochemistry-based approach has revealed that we are just scratching the proverbial surface of the roles played by redox carriers in the pathways of CO2 reduction.

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Abstract 2498

Ammonification: How to go from nitrite to ammonia with one enzyme

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Nitrate and nitrite are serious contaminants that can lead to eutrophication of waterways. Certain organisms, however, can convert nitrate and nitrite to ammonium via the dissimilatory nitrate reduction to ammonium (DNRA) pathway. One of the enzyme complexes capable of performing DNRA is composed of two proteins: NrfH, a membrane-bound quinol oxidase, and its redox partner NrfA, a soluble periplasmic nitrite ammonifier. NrfA catalyzes the six-electron, eight-proton reduction of nitrite to ammonium and helps retain nitrogenous nutrients in the soil. This project aims to (i) ascertain the precise enzymatic mechanism of NrfA and (ii) elucidate the strategy for the storage and flow of electrons. We are employing a synergistic combination of biochemical, kinetic, spectroscopic, and electrochemical methods to trap and interrogate reaction intermediates to accomplish these objectives. We are focusing our studies on the NrfA enzyme from *Geobacter lovleyi*, a DNRA bacterium identified for its environmental relevance. We developed a heterologous expression system for *G. lovleyi* NrfA in *Shewanella oneidensis* MR-1. We determined that *G. lovleyi* NrfA has a calculated rate constant (k_{cat}) and Michaelis constant (K_M) that agree with published values. We solved the crystal structure of the *G. lovleyi* NrfA at 2.55 Å resolution. The structure revealed a crystallographic dimer and heme configuration consistent with other structurally characterized NrfA homologs. While the crystallographic data are consistent with previously characterized NrfA homologs, dynamic light scattering provided evidence that purified *G. lovleyi* NrfA is a monomer in solution at concentrations as high as ~300 mM. This could either mean that NrfA is not a functional dimer or that it requires its natural redox partner NrfH to dimerize. Surprisingly, the *G. lovleyi* NrfA crystal structure revealed an arginine residue in the active site region that would otherwise be occupied by Ca^{2+} in other structurally characterized NrfA homologs. Site-directed mutagenesis of this arginine to lysine, glutamine, or alanine resulted in variants that are no more than 3% active compared to the WT enzyme. We constructed a phylogenetic tree from 445 aligned amino acid sequences and identified 4 separate nodes marking the emergence of Arg-containing NrfA enzymes. This finding suggests a novel subclass of Arg-containing, Ca^{2+}-independent NrfA enzymes. From the phylogenetic analysis, we inferred that the emergence of Ca^{2+}-independent NrfA proteins may allow these bacteria to ammonify nitrate and nitrite in environments where Ca^{2+} is limited or tightly controlled. Together with our collaborators, we probed electron movement through this pentaheme enzyme. Using a combination of electrochemical experimetns and EPR analysis, we demonstrated that hemes 4

and 5 of NrfA are reduced first (before the active site heme 1) and likely serve the purpose of an electron storage unit within the protein. We investigated the role of the central heme 3 by generating an H108M NrfA variant which positively shifted the reduction potential of heme 3 (from −226 to +48 mV). The H108M mutation significantly impacts both the distribution of electrons within the pentaheme scaffold and the hemes' reduction potential, reducing the enzyme's catalytic activity to 1% compared to that of the wild-type enzyme. We propose that this is due to heme 3′s important role as an electron gateway in native NrfA.

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Abstract 2516

Evidence of substrate binding and product release via belt-sulfur mobilization of the nitrogenase cofactor

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Molybdenum nitrogenase catalyzes the ambient reduction of N2 to NH3 at the M-cluster, a complex cofactor that comprises two metal-sulfur sub-cubanes ligated by an interstitial carbide and three belt-sulfides. A recent crystallographic study suggests binding of N2 via displacement of the belt-sulfur(s) of the M-cluster upon turnover. However, direct proof of N2 binding and belt-sulfur mobilization during catalysis remains elusive. Here we show that N2 is captured on the M-cluster via electron- and sulfur-depletion, and that the N2-captured state is catalytically competent in generating NH3. Moreover, we demonstrate that product release occurs only when sulfite is supplied along with a reductant, that sulfite is inserted as sulfide into the belt-sulfur-displaced positions, and that there is a dynamic in-and-out of belt-sulfurs during catalysis.[1] Together, these results establish the mobilization of cofactor belt-sulfurs as a crucial, yet overlooked, mechanistic element of the nitrogenase reaction. [1] Lee CC, Kang W, Jasniewski AJ, Stiebritz MT, Tanifuji K, Ribbe MW, Hu Y. Nat Catal. 2022 May;5(5):443–454. doi: 10.1038/s41929-022-00782-7.

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Abstract 2522

How anaerobic microbes make and break methane: mechanism of Methyl-Coenzyme M Reductase

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One of the largest threats facing humanity is the increasing global concentration of greenhouse gases carbon dioxide and methane in the atmosphere, affecting rising sea levels, ocean acidification, and severe climate conditions. Methanogenic archaea are responsible for the production of 1 billion tons of methane per year and account for nearly all methane found on earth. Methyl Coenzyme M Reductase (MCR), the key enzymatic catalyst in the anaerobic synthesis of methane (Equation 1), also catalyzes the reverse reaction - the anaerobic oxidation of methane (AOM). This protein is unique to methanogens and anaerobic methane oxidizers. MCR is one of the eight known Ni enzymes that catalyze the utilization and/or production of gases that play important roles in the global biological carbon cycle. MCR is the only enzyme containing a nickel tetrapyrrole (F430). It contains novel post-translational modifications in amino acid residues located at the surface of a 50 Å-long channel that accommodates the two substrates or the products. Spectroscopic and kinetic studies have demonstrated that the active state of the enzyme contains a low-valent and highly oxygen- and redox-sensitive Ni(I) state and that the metal ion appears to traverse three oxidation states (1+, 2+ and 3+) during catalysis. We will describe recent spectroscopic and structural studies that have identified a methyl radical at the transition state for methane synthesis. Other studies, including serial crystallography and X-ray spectroscopy, indicate that the active Ni(I) form of MCR exhibits significant active site rearrangements relative to the inactive Ni(II) form observed in all published structures. These results are leading to an enhanced understanding of how microbes make and break methane. CH3-SCoM + CoBSH → CH4 + CoB-SS-CoM $\Delta G0' = -30$ kJ/mol Equation 1.

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Abstract 2523

Biochemical Characterization and Mutational Analysis of the Catalytic Domain of E2 Conjugation Enzyme, Ubc9 from *Plasmodium falciparum*

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Hina Bharti, Alo Nag

Malaria is one of the serious parasitic diseases worldwide, caused by the protozoan *P. falciparum*. To ensure their survival, malaria parasites use sophisticated mechanisms to engage and infiltrate host cells. Apart from the clinical manifestations of the disease, the principle of molecular mechanism causing propagation of the parasite is largely unknown. The post-translational machinery including SUMOylation is believed to control numerous vital activities of the parasite. The functioning of the SUMO conjugating enzyme, Ubc9 has been extensively studied and proven to play multitude functions in higher eukaryotes. However, PfUbc9 has not been investigated in detail. In the present work, we have attempted to understand the dynamics of the enzyme active site and reported the analyses of previously uncharacterized mutations in PfUbc9 active site. The crystal structure of the native enzyme provided a framework for molecular docking with the SUMOylation substrate, RanGAP1. Subsequently, the bioinformatic analyses of PfUbc9 through various approaches led us to predict the catalytically important residues of PfUbc9. Using Fluorescence and Circular Dichroism Spectroscopy, we have investigated the effect of mutation on protein structure and folding. Finally, we utilised SUMO conjugation assay to carry out the functional analysis of the mutated PfUbc9 proteins. Our study highlights the dynamics of SUMOylation machinery of *P. falciparum*. For the first time, we dissected the differential structure-function properties of the host and parasite Ubc9 presenting a comparison of the selected catalytically important residues between PfUbc9 and HsUbc9. Interestingly, we could also demonstrate that swapping the amino acid residue of PfUbc9 residue with the one present in HsUbc9 abolished the activity of the parasite enzyme. To conclude, this study of the structure analysis complemented with biochemical characterization provides a novel unprecedented data of the crucial amino acids in PfUbc9 protein. Our research findings lay a foundation for considering PfUbc9 as a potent antimalarial therapeutic target.

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Abstract 2544

Radical SAM aminomutases catalyzing beta-amino acid biosynthesis for salt tolerance in methanogenic archaea

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Radical *S*-adenosylmethionine (SAM) enzymes catalyze a variety of complex reactions in diverse biochemical pathways across all domains of life. Interestingly, the genomes of methanogenic archaea (methanogens) encode many radical SAM enzymes with underexplored or completely unknown functions. Here, we describe the biochemical characterization of a methanogenic lysine 2,3-aminomutase involved in the biosynthesis of N(epsilon)-acetyl-β-lysine, a key osmolyte required for methanogens to survive in high salinity environments. We further report the function of a putative radical SAM aminomutase found in the genomes of some methanogens that contains a ∼160 amino acid extension at its N-terminus. Bioinformatic analysis of possible substrate binding residues suggested a function as a glutamate 2,3-aminomutase, which was confirmed through heterologous expression experiments and analysis of β-glutamate in methanogens containing or lacking the gene of interest. β-glutamate has been known to serve as an osmolyte in some methanogens for a long time, but its biosynthetic origin remained unknown until now. Thus, this work defines the biosynthetic routes for β-lysine and β-glutamate in methanogens and expands the importance and diversity of radical SAM enzymes in the archaeal domain of life.

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Abstract 2564

Substrate specificity of homodimeric and heterodimeric nucleoside hydrolase complexes from *Arabidopsis thaliana*

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Purine catabolism in plants provides a metabolic route for nitrogen remobilization from nitrogen-rich purine nucleobases, which is crucial for plant physiology. Cytosolic nucleoside hydrolase (NSH) participates in the purine degradation pathway by cleaving the N-glycosidic bond between nucleobase and ribose. In *Arabidopsis thaliana*, cytosolic NSH1 and its homolog NSH2 were characterized to form either of homodimeric NSH1 complex or heterodimeric NSH1–NSH2 complex. Furthermore, heterodimeric NSH1–NSH2 complex exhibited a substrate specificity distinct from that of homodimeric NSH1–NSH1 complex, suggesting a unique role of homodimeric or heterodimeric complex in purine catabolism. For example, heterodimeric NSH1–NSH2 complex has a higher specificity against xanthosine and inosine which serve as two major intermediates for purine catabolism in Arabidopsis. However, biochemical and structural basis remains unknown for differences in the substrate specificity between homodimeric and heterodimeric complex, mainly due to failure in purifying those two complexes. Recently, we were successful in expressing and purifying the two complexes. In this presentation, we will address our progresses on the structural and biochemical study of NSH complexes which play crucial roles in purine catabolism.

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Abstract 2570

Structures and kinetics of *Thermotoga maritima* MetY reveal insights into the predominant route for methionine biosynthesis in bacteria

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Petr Pachl, James McKellar, Maria Selmer, Christopher Squire, Wayne Patrick

Methionine is essential to all life on Earth. As the primary intracellular carrier of methyl groups and precursor to S-adenosyl methionine, it is central to the metabolic network. It also has crucial roles in the initiation of protein translation and protein folding, stability, and function. Bacterial methionine biosynthesis can occur either by a two-step trans-sulfurylation or a single-step direct sulfurylation route. The sulfurylation enzymes are all homologues and utilise the coenzyme pyridoxal 5′-phosphate (PLP). The trans-sulfurylation enzymes have been extensively characterized. However, across the bacterial phylogenetic tree, direct sulfurylation is the predominant route for methionine biosynthesis. Direct sulfurylation, converting O-acetylhomoserine and bisulfide to homocysteine, is catalyzed by MetY. However, despite the widespread distribution of this enzyme, it remains comparatively understudied. To address this knowledge gap, we have characterized MetY from the thermophile *Thermotoga maritima* (TmMetY). We found that TmMetY can use O-succinylhomoserine as an alternative substrate, but with ∼1000-fold lower efficiency relative to the native substrate, O-acetylhomoserine. This is interesting as O-succinylhomoserine is the substrate used by the MetY homologue, MetB, which catalyzes one of the trans-sulfurylation reactions. To investigate the structural determinants of substrate specificity by TmMetY, we collected X-ray diffraction data of TmMetY crystals alone or soaked with the substrate O-acetylhomoserine. In the presence of the substrate we fortuitously captured a β,γ-unsaturated ketimine reaction intermediate. This intermediate is identical to that found in the catalytic cycle of MetB, confirming that the two homologues use the same mechanism. By comparing the structures of TmMetY and MetB, we identified Arg270 as a critical determinant of substrate specificity. Its position disfavours the binding of O-succinylhomoserine in TmMetY and ensures a strict specificity for bisulfide as the second substrate, by occluding the larger MetB substrate, cysteine. Overall, this work illuminates the subtle structural mechanisms by which PLP-dependant homologous enzymes can effect different catalytic – and therefore metabolic – outcomes.

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Abstract 2589

Probing alternative substrate binding and cleavage of class A sortases

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Jeanine Amacher

Sortases are an enzyme family of cysteine transpeptidases, consisting of classes A-F, which are found in the cell wall of gram-positive bacteria. They perform a critical role in ligating proteins to the cell wall, including those that are responsible for cell adhesion, immune evasion, host cell invasion, and nutrient acquisition. Sortases are transpeptidase enzymes, and their catalytic mechanism involves first recognizing and cleaving a target sequence, followed by ligation with a second nucleophilic substrate. The pathogenic roles of class A sortases make them enzymes of considerable interest for therapeutic interventions. Class A sortases (SrtAs) are also an attractive tool for protein engineering strategies that have enabled sortase mediated ligation or sortagging strategies. Their utility as a bioengineering tool results from functions that allow ligation of proteins to the cell wall. Class A sortases accomplish this by recognizing a cell wall sorting signal, LPXTG, and cleaving the amide bond between the threonine and glycine residues, at which point a glycine nucleophile will bind to the cleaved LPXT- side of the reaction. Research conducted by our collaborator John Antos (Western Washington University) found that some of these class A sortases could cleave and perform the ligation between the Gly position and another residue, if the Gly is substituted by a large hydrophobic residue, e.g., Leu, Phe, and/or Tyr. Following up on this research, we wondered which structural motifs and interactions play a role in the binding and alternative cleavage of substrates, given the known propensity of enzymes to perform reactions with high specificity. For example, *Streptococcus pyogenes* SrtA shows almost exclusive alternative cleavage with the sequence LPXTLG, but is less selective for LPXTFG or LPXTYG. In contrast, *Listeria monocytogenes* SrtA does not recognize LPXTLG, but alternatively cleaves LPXTYG to a high degree. To study this, we created *S. pyogenes/ L. monocytogenes* chimeric loop swap proteins and point mutations, and ran HPLC/mass spectrometry cleavage assays in order to tease apart how this alternative cleavage may occur. Of particular interest is the H143 residue adjacent to the catalytically active H142, which is highly conserved amongst constructs capable of binding and performing alternative cleavages. Overall, we find that indeed, specific residues in these proteins are responsible for alternative cleavage specificity. This work may provide new and exciting applications for sortase-mediated ligation strategies.

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Abstract 2611

Rapid Reaction Kinetics & characterization of FAD in the reductive and oxidative half reactions of bcd in *Megasphaera elsdenii*

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Derek Nguyen, Russ Hille

In order to understand the mechanism of bifurcation in the crotonyl-CoA-dependent NADH:ferredoxin oxidoreductase from the bacterium *M. elsdenii* and determine why the reaction proceeds in such high fidelity and tight observed coupling, we have taken to analyzing each individual component of the bifurcating system above. Previous work has focused on the EtfAB (electron transferring flavoprotein) component both fully replete of both FAD cofactors and depleted of the electron transferring FAD. Current work focuses on the reductive behavior of bcd (butyryl-CoA dehydrogenase) with sodium dithionite, NADH from EtfAB and butyryl-CoA and the oxidative behavior with crotonyl-CoA. UV-Visible spectroscopy was used to characterize the various forms of the bcd FAD; the oxidized quinone state (FAD), reduced hydroquinone (FADH-), the 1 electron semiquinone states (FADH• and FAD•-), the substrate bound charge transfer complexes (bcdred:crotonyl-CoA and bcdox:butyryl-CoA). Stopped-flow spectroscopy was utilized in order to gain rapid reaction kinetics data and determine the experimental rate constants of reduction/oxidation and the apparent binding of substrate. Lastly, electron paramagnetic resonance spectroscopy (EPR) was employed in order to quantify and qualify the accumulation of the semiquinone and its state. The bcd FAD is able to facilitate both 1 and 2 electron transfers as seen when reduced with sodium dithionite. We observe the accumulation of the neutral semiquinone FADH- as confirmed via EPR that is pH dependent. When the enzyme is titrated catalytically, i.e., NADH reduced EtfAB we do not observe any differences than when it is reduced with sodium dithionite in the presence of EtfAB. We also observed the presence of charge transfer complexes upon binding of substrate and a change in the ionization state of the semiquinone to that of the anionic form FAD•- upon said binding. Investigation of the pre steady state reaction of bcd and EtfAB yielded surprising results. The rate of electron transfer from a pre reduced EtfAB was independent of protein concentration and slow (2 s-1). The reoxidation of reduced bcd was faster yet remained independent of [crotonyl-CoA] (30 s-1). Reduction when reacting with butyryl-CoA was the fastest (1400 s-1) but was dependent on [butyryl-CoA]. Although the rate of reduction from butyryl-CoA is orders of magnitude faster, the extent of reduction is significantly less and cannot be overcome via mass action. From these experiments we observe both 2 and 1 electron processes along the high potential pathway of the bifurcating system. This behavior corresponds to other bifurcating systems that have an obligate 1 electron acceptor along the pathway in the form of iron sulfur clusters and the nature of the non-bifurcating Etfs in which it is a single electron transfer to the final electron acceptors.

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ASBMB

Abstract 2620

Opposing regulation of METTL11A by its family members METTL11B and METTL13

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N-terminal protein methylation is a post-translational modification (PTM) that influences a variety of biological processes by regulating protein stability, protein-DNA interactions, and protein-protein interactions. Although significant progress has been made in understanding the biological roles of this PTM, we still do not completely understand how the methyltransferases that place it are regulated. A common mode of methyltransferase regulation is through complex formation with close family members, and we have previously shown that the N-terminal trimethylase METTL11A (NRMT1/NTMT1) is activated through binding of its close homolog METTL11B (NRMT2/NTMT2). It has also recently been reported that METTL11A co-fractionates with a third METTL family member METTL13, which methylates both the N-terminus and lysine 55 (K55) of eukaryotic elongation factor 1 alpha (eEF1A). Here, we combine co-immunoprecipitation, mass spectrometry, and *in vitro* methyltransferase assays to confirm a regulatory interaction between METTL11A and METTL13 and show that, while METTL11B is an activator of METTL11A, METTL13 inhibits METTL11A activity. This is the first example of a methyltransferase being opposingly regulated by different family members. Similarly, we find that METTL11A promotes the K55 methylation activity of METTL13, but inhibits its N-terminal methylation activity. We also find that catalytic activity is not needed for these regulatory effects, demonstrating new, non-catalytic functions for METTL11A and METTL13. Finally, we show METTL11A, METTL11B, and METTL13 can complex together, and when all three are present, the regulatory effects of METTL13 take precedence over those of METTL11B. These findings provide a better understanding of the regulation of N-terminal methylation, and suggest a model where these methyltransferases can serve in both catalytic and non-catalytic roles.

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Abstract 2628

Characterization of a FeoC-like protein in *Methanococcus voltae*

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Emily Dieter, William Broderick, Joan Broderick

The acquisition, deployment and use of iron by methanogenic archaea is truly remarkable, largely due to methanogen's relatively significant reliance on iron. In fact, one well studied methanogen strain contains roughly 15 times the number of [Fe-S] clusters per mg of protein than a comparable amount from *E. coli* cells. Recent work has shown that model methanogens can source iron and sulfur from pyrite (FeS2), the most abundant sulfide mineral on Earth. Although pyrite was previously thought to be largely biologically unavailable, *Methanococcus voltae* can reductively dissolve pyrite at relatively low temperatures and pressure in anoxic environments, a feat previously only synthetically achievable at very high temperatures and pressures (>90°C and >15 bar H2). Following the dissolution of pyrite, [Fe-S] cluster products are trafficked into the cell. Analysis of over 300 methanogenic genomes has shown the importance of the Feo system, which facilitates internal ferrous iron transport, to these methanogens. The Feo operon is composed of three genes; FeoA, FeoB and FeoC. FeoA and FeoB are seen to be conserved across most methanogen genomes. FeoC, however, has only been found in γ-proteobacteria. Through shotgun proteomics, we have identified a protein that shows structural and genomic homology to FeoC that is upregulated in *M. voltae* when the organism is grown on pyrite. The role of FeoC in the Feo system is not well understood, but it is believed to aid in the detection and monitoring of iron acquisition into the cell. We have overexpressed and purified this protein, and are currently working on further characterization, including reconstituting iron clusters, analyzing through EPR spectroscopy, and investigating protein-protein interactions with FeoA and FeoB to further elucidate and define the role of this FeoC homolog in the Feo system in *Methanococcus voltae*.

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Abstract 2648

Can we predict coenzyme Q deficiency in patients? The case of COQ5, a C-methyltransferase in coenzyme Q biosynthesis

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Kendall Houk, Steve Clarke, Catherine Clarke

The COQ5 gene encodes an S-adenosylmethionine (AdoMet)-dependent C-methyltransferase involved in biosynthetic pathway of coenzyme Q (CoQ) in humans and in the yeast *Saccharomyces cerevisiae*. Coq5-deficient yeast are respiratory-deficient and hypersensitive to polyunsaturated fatty acid treatment, as a result of two central roles of CoQ and its reduced form (CoQH2): mitochondrial electron carrier, and chain-terminating antioxidant protecting against lipid peroxidation. Likewise, a defective CoQ biosynthetic pathway in humans can cause a wide array of illnesses, including cardiovascular, kidney, and neurodegenerative disorders, through a condition known as primary CoQ deficiency. Here, we focus on previously reported human COQ5 missense single nucleotide variants (SNVs), most of which have unknown clinical significance. We identified SNVs of potential clinical relevance using a combination of available structural, functional, and sequence information in the human COQ5 polypeptide and its close homologs. Using yeast- and *Escherichia coli*-based *in vitro* and *in vivo* studies, we plan to examine the effects of these SNVs on catalytic activity, CoQ biosynthesis, and ability to assemble or stabilize the CoQ synthome, a high molecular mass complex required for CoQ biosynthesis. These findings will be further validated through density functional theory (DFT) calculations and molecular dynamics (MD) simulations performed on the wild type and predicted mutant yeast structures, to identify differences in the energetics of catalysis and ligand binding as well as elucidate the catalytic mechanism. Our results will shed light on the structure-and-function relationship of the methyltransferase Coq5/COQ5 and its role in maintaining the CoQ synthome. By combining the power of biochemical and computational approaches, our work presents a strategy for facilitating the screening and diagnosis of primary CoQ deficiency as well as other single-gene disorders where structural information is limited.

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Abstract 2668

Characterization of novel carboxylesterase protein function using the BASIL* curriculum

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Elizabeth Abboud

As the fundamental "workhorse" molecules of life, proteins can be found in every cell of every tissue. They appear in a vast variety of structures, each performing unique functions. Studying protein function is essential to understanding the molecular process of life along with countless biological disorders. The goal of this project was to characterize a novel protein of unknown function using computational analysis, protein purification and enzymatic activity assays. The plasmid was cultured in *E. coli* under ampicillin selection, purified and sequenced to verify identity. The BLAST alignment revealed the protein of interest to be a member of the carboxylesterase superfamily. Protein expression was induced with IPTG and harvested via B-PER® Bacterial Protein Extraction Reagent. Total protein was spectrophotometrically quantified via Bradford Protein Assay. Crude lysate proteins were resolved on 4–20% SDS-PAGE gels and results confirmed that the lysate contained a 28 kDa protein, which is consistent with the expected size of the target protein. The project is ongoing; the next steps include protein purification using immobilized metal affinity chromatography (IMAC) and functional identification via enzyme activity assay. *This project was accomplished in accordance with the protocols recommended by The Biochemistry Authentic Scientific Inquiry Laboratory Community (The BASIL Biochemistry Curriculum), basilbiochem.org, January 17, 2023. Ashley Ringer McDonald, Herbert J. Bernstein, S. Colette Daubner, Jonathan D. Dattelbaum, Anya Goodman, Bonnie L. Hall, Stefan M. Irby, Julia R. Koeppe, Jeffrey L. Mills, Stephen A. Mills, Suzanne F. O'Handley, Michael Pikaart, Rebecca Roberts, Arthur Sikora, Paul A. Craig.

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ASBMB

Abstract 2672

Purification and characterization of AKR1C18 from Peromyscus demonstrates it is an enzyme that catalyzes the conversion of progesterone to 20α-hydroxyprogesterone

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Natalie Niepoth, Andrés Bendesky

Progesterone is an endogenous steroid hormone that has important roles in establishing and maintaining pregnancy in mammals. One way in which the levels of progesterone are regulated is through enzymes that convert progesterone into forms with lower affinity for the Progesterone Receptor. Members of the aldo-keto reductase (AKR) superfamily of enzymes utilize NAD(P)H to catalyze the reduction of carbonyl groups to generate primary and secondary alcohols. AKR enzymes act on diverse substrates, including ketosteroids such as progesterone. We found that species of the rodent genus Peromyscus differ strikingly in the levels of AKR1C18 in their adrenal glands. The house mouse and brown rat homologs of AKR1C18 catalyze the 20α reduction of progesterone into 20α-hydroxyprogesterone. However, there are amino acid differences between those species and Peromyscus AKR1C18, so we tested if its 20α-hydroxysteroid dehydrogenase activity was conserved. By using enzymatic assays and reverse-phase high performance liquid chromatography linked to UV detection (RP-UV/HPLC), we show that purified recombinant Peromyscus AKR1C18 also catalyzes the reduction of progesterone to 20α-hydroxyprogesterone with NADH as a cofactor. This finding has potential implications for processes regulated by progesterone, including parental care.

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Abstract 2676

SARS-CoV-2 Nsp13 Catalytic Efficiency is Regulated by ATP: Mg2+ Stoichiometry and Functional Cooperativity Among Nsp13 Molecules

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Joshua Sommers, Lorin Loftus, Martin Jones, III, Rebecca Lee, Adaira Dumm, Robert Brosh, Jr

Coronavirus disease 19 (COVID-19) is a highly contagious and lethal disease caused by the SARS-CoV-2 positive-strand RNA virus. Nonstructural protein 13 (Nsp13) is the highly conserved ATPase/helicase required for replication of the SARS-CoV-2 genome which allows for the infection and transmission of COVID-19. We biochemically characterized the purified recombinant SARS-CoV-2 Nsp13 helicase protein expressed using a eukaryotic cell-based system and characterized its catalytic functions, focusing on optimization of its reaction conditions and assessment of functional cooperativity among Nsp13 molecules during unwinding of duplex RNA substrates. These studies allowed us to carefully determine the optimal reaction conditions for binding and unwinding various nucleic acid substrates. Previously, ATP concentration was suggested to be an important factor for optimal helicase activity by recombinant SARS-CoV-1 Nsp13. Apart from a single study conducted using fixed concentrations of ATP, the importance of the essential divalent cation for Nsp13 helicase activity had not been examined. Given the importance of the divalent metal ion cofactor for ATP hydrolysis and helicase activity, we assessed if the molar ratio of ATP to Mg2+ was important for optimal SARS-CoV-2 Nsp13 RNA helicase activity. We determined that Nsp13 RNA helicase activity was dependent on ATP and Mg2+ concentrations with an optimum of 1 mM Mg2+ and 2 mM ATP. Next, we examined Nsp13 helicase activity as a function of equimolar ATP:Mg2+ ratio and determined that helicase activity decreased as the equimolar concentration increased, especially above 5 mM. We determined that Nsp13 catalytic functions are sensitive to Mg2+ concentration suggesting a regulatory mechanism for ATP hydrolysis, duplex unwinding, and protein remodeling, processes that are implicated in SARS-CoV-2 replication and proofreading to ensure RNA synthesis fidelity. Evidence is presented that excess Mg2+ impairs Nsp13 helicase activity by dual mechanisms involving both allostery and ionic strength. In addition, using single-turnover reaction conditions, Nsp13 unwound partial duplex RNA substrates of increasing double-stranded regions (16–30 base pairs) with similar kinetic efficiency, suggesting the enzyme unwinds processively in this range under optimal reaction conditions. Furthermore, we determined that Nsp13 displayed sigmoidal behavior for helicase activity as a function of enzyme concentration, suggesting that functional cooperativity and oligomerization are important for optimal activity. The observed functional cooperativity of Nsp13 protomers suggests the essential

coronavirus RNA helicase has roles in RNA processing events beyond its currently understood involvement in the SARS-CoV-2 replication-transcription complex (RTC), in which it was suggested that only one of the two Nsp13 subunits has a catalytic function, whereas the other has only a structural role in complex stability. Altogether, the intimate regulation of Nsp13 RNA helicase by divalent cation and protein oligomerization suggests drug targets for modulation of enzymatic activity that may prove useful for the development of novel anti-coronavirus therapeutic strategies.

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Abstract 2677

The SARS-CoV-2 Nsp13 Helicase Required for Coronavirus Replication Interacts with Structured Nucleic Acids in a Strand-Specific Manner and Catalyzes a Novel Protein-RNA Remodeling Activity Implicated in the Proofreading Mechanism of the Replication-Transc

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a single-stranded, positive-sense RNA virus responsible for COVID-19, requires a set of virally encoded nonstructural proteins that compose a replication-transcription complex (RTC) to replicate its 30 kilobase genome. One such nonstructural protein within the RTC is Nsp13, a highly conserved molecular motor ATPase/helicase. Upon purification of the recombinant SARS-CoV-2 Nsp13 protein expressed using a eukaryotic cell-based system, we biochemically characterized the enzyme by examining its catalytic functions, nucleic acid substrate specificity, and putative protein-nucleic acid remodeling activity. We determined that Nsp13 preferentially interacts with single-stranded (ss) DNA compared to ssRNA during loading to unwind with greater efficiency a partial duplex helicase substrate. The binding affinity of Nsp13 to nucleic acid was confirmed through electrophoretic mobility shift assays (EMSA) by determining that Nsp13 binds to DNA substrates with significantly greater efficiency than RNA. These results demonstrate strand-specific interactions of SARS-CoV-2 Nsp13 that dictate its ability to load and unwind structured nucleic acid substrates. We next determined that Nsp13 catalyzed unwinding of double-stranded (ds) RNA forked duplexes on substrates containing a backbone disruption (neutrally charged polyglycol linker (PGL)) was strongly inhibited when the PGL was positioned in the 5′ ssRNA overhang, suggesting an unwinding mechanism in which Nsp13 is strictly sensitive to perturbation of the translocating strand sugar-phosphate backbone integrity. Furthermore, we demonstrated for the first time the ability of the coronavirus Nsp13 helicase to disrupt a high-affinity nucleic acid-protein interaction, i.e., a streptavidin tetramer bound to biotinylated RNA or DNA substrate, in a uni-directional manner and with a preferential displacement of the streptavidin complex from biotinylated ssDNA versus ssRNA. In contrast to the poorly hydrolysable ATP-gamma-S or non-hydrolysable AMP-PNP, ATP supports Nsp13-catalyzed disruption of the nucleic acid-protein complex, suggesting that nucleotide binding by Nsp13 is not sufficient for protein-RNA disruption and the chemical energy of nucleoside triphosphate hydrolysis is required to fuel remodeling of protein bound to RNA or DNA. Our results build upon structural studies of the SARS-CoV-2 RTC in which it was

suggested that Nsp13 pushes the RNA polymerase (Nsp12) backward on the template RNA strand. Experimental evidence from our studies demonstrate that Nsp13 helicase efficiently remodels a large high affinity protein-RNA complex in a manner dependent on its intrinsic ATP hydrolysis function. We proposed that this novel biochemical activity of Nsp13 is relevant to its role in SARS-CoV-2 RNA processing functions and replication. It was proposed that Nsp13 facilitates proof-reading during coronavirus replication when a mismatched base is inadvertently incorporated into the SARS-CoV-2 genome during replication to reposition the RTC so that the proof-reading nuclease complex (Nsp14-Nsp10) can gain access and remove the nascently synthesized nucleotide to ensure polymerase fidelity. Our findings implicate a direct catalytic role of Nsp13 in protein-RNA remodeling during coronavirus genome replication beyond its duplex strand separation or structural stabilization of the RTC, yielding new insight into the proof-reading mechanism.

This work was supported by the Intramural Training Program, National Institute on Aging (NIA), NIH, and a Special COVID-19 Grant from the Office of the Scientific Director, NIA, NIH.

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Abstract 2680

RECON Syndrome Helicase is Potently Blocked Upon Encountering a Single Mono-ribonucleotide in the Translocating Strand of a Forked Duplex DNA Substrate

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Ribonucleotides may be mis-incorporated into the genome during cellular DNA replication, resulting in genomic instability and mutations which underlie certain hereditary diseases such as Aicardi-Goutieres syndrome. Although DNA repair mechanisms that replace a mono-ribonucleotide with the correct deoxyribonucleotide are known to involve nucleolytic incision by RNaseH1/2 or Topoisomerase I at the site of the mis-incorporated nucleotide, it is unclear if other DNA binding proteins or metabolizing enzymes affect recognition/incision of the helix-distorting lesion. One class of enzymes of particular interest is helicase/translocases which i) have the ability to scan the genome for structural perturbations, ii) play instrumental roles in DNA transactions during replication, DNA repair and transcription, and iii) are mutated in chromosomal instability disorders prone to hyper-mutation. This prompted us to assess the effects of a site- and strand-specific uridylate (rU) or cytidylate (rC) harbored within the double-stranded DNA segment of a forked partial duplex substrate on unwinding catalyzed by biologically relevant helicases implicated in replication or DNA repair. The ring-like multi-subunit DNA helicases MCM and Twinkle implicated in replication of the nuclear and mitochondrial genomes, respectively, are fully tolerant of a rU or rC, whereas notable DNA repair helicases are sensitive to even a single ribonucleotide nested within the duplex of the helicase substrate. The RECQ helicases WRN and RECQL1 mutated in the premature aging disorders Werner syndrome and RECON syndrome, respectively, are strongly inhibited by a rU or rC positioned in the helicase-translocating strand even under multi-turnover conditions; however, only modestly affected by the rC and unaffected by the rU lesion in the non-translocating strand. Under single-turnover conditions, the kinetics of unwinding a forked duplex substrate with a single rU by RECQL1 is inhibited nearly 4-fold. To probe the mechanism further, we tested a clinically relevant and catalytically compromised RECQL1-A459S mutant protein and found it to be profoundly inhibited by the rU substrate. Both RECQL1 and WRN were sequestered by the forked duplex harboring the rU in the translocating strand, suggesting that the helicase is trapped by the lesion upon encountering it. Consistent with this, preincubation of RECQL1 in the presence of ATP in helicase-active reaction conditions with the forked duplex substrate containing the translocating strand rU, but not the non-translocating strand rU or lacking rU altogether, resulted in protection of the partial duplex substrate from cleavage by the restriction endonuclease HaeIII. Taken

together, these results suggest that even a single ribonucleotide can affect genomic transactions of certain helicases by inhibiting strand separation activity or becoming trapped at the site of a mono-ribonucleotide, which may serve to signal DNA repair machinery for localization and subsequent action.

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Abstract 2683

Regulation of malarial phosphatidylserine decarboxylase proenzyme processing by phospholipids

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Phosphatidylserine decarboxylases (PSDs) are important enzymes in phospholipid synthesis and membrane biogenesis in many organisms. PSDs catalyze the decarboxylation of PS to form PE, an essential structural phospholipid found in all membranes. Processing of a malarial PSD proenzyme into its active a- and b- subunits is by an auto-endoproteolytic mechanism regulated by anionic phospholipids with PS serving as an activator, and PG, PI, and PA acting as inhibitors. We used solid phase lipid binding, liposome binding assays and surface plasmon resonance (SPR) to examine the binding specificity of a processing-deficient Plasmodium PSD (PkPSDS308A) mutant enzyme, and demonstrated that the PSD proenzyme binds strongly to PS and PG but not to PE and PC. The equilibrium dissociation constants of PkPSD with PS and PG were 80.4 nM and 66.4 nM, respectively. The interaction of PSD with PS is inhibited by calcium implying binding via ionic interactions. Peptide mapping identified polybasic amino acid motifs in the proenzyme responsible for binding to PS. Altogether, the data demonstrate that malarial PSD proenzyme maturation is regulated through a strong physical association between PkPSD proenzyme and anionic lipids. Inhibition of the specific interaction between the proenzyme and the lipids can provide a novel mechanism to disrupt PSD enzyme activity.

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Topic Category Genomics, Glycomics, Proteomics and Metabolomics

Abstract 1184

Utilization of keratinous chicken feathers for the extracellular production of keratinase by Bacillus sp. CSK2: keratinolytic enzyme characterization

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Uchechukwu Nwodo

Feathers are the most abundant waste materials generated from processing poultry birds and present major concerns to the environmentalists due to their recalcitrant nature. Production of keratinolytic enzymes utilizing cheaply available keratinous biomass is highly attractive from both ecological and biotechnological perspectives. Consequently, wild isolate - Bacillus sp. CSK2 was grown on chicken feathers for keratinase biosynthesis. The physicochemical conditions influencing keratinase production by Bacillus sp. CSK2 was optimized, and the keratinase was characterized via biochemical and molecular approaches. The highest enzyme activity of 1539.09 ± 68.14 U/mL was obtained after 48 h of incubation with optimized conditions consisting of chicken feathers (7.5 g/L), maltose (2.0 g/L), initial fermentation pH (5.0), incubation temperature (30°C) and agitation speed (200 rpm). The keratinase showed optimal catalytic efficiency at pH 8.0 and temperature (60–80° C). The keratinase thermostability was remarkable with half life time of above 120 min at 70°C. Enzymatic biocatalysis was inhibited by ethylenediaminetetraacetic acid and 1,10-phenanthroline. However, keratinase activity was enhanced by 2-mercaptoethanol, dimethyl sulfoxide, tween-80, but showed varied degree loss of activity in the presence of metal ions; with Al3+ and Fe3+ exerting the highest inhibition. Upon treatment with laundry detergents, the following keratinase residual activities were achieved: 85.19 ± 1.33% (Sunlight), 90.33 ± 5.95% (Surf), 80.16 ± 2.99% (Omo), 99.49 ± 3.11% (Ariel), and 87.19 ± 0.26% (Maq). Furthermore, the amino acid sequence of the keratinase showed some variation, in the sequence residues, compared to related sequences from the database. The computed theoretical isoelectric point, instability and aliphatic indices were 9.23, 26.85 and 75.82, respectively. Bacillus sp. CSK2 keratinase displayed some unique attributes. The subcellular localization prediction suggested that the novel keratinase is a secretory protein, and that would be an attraction if the industrial and biotechnological potential of the enzyme are further explored.

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Abstract 1366

Compounds enhancing poly(3-hydroxybutyrate) storage: cell membrane interfering agent 2-phenylethanol combined with nitrogen starvation triggered poly(3-hydroxybutyrate) accumulation in cyanobacteria Synechocystis sp. PCC6803 and Anabaena sp

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Various cyanobacteria can increase bioplastic poly(3-hydroxybutyrate) (PHB) accumulation under nitrogen-limited cultivation as carbon reserve. Genetic engineering has been applied to modify metabolism to stimulate PHB biosynthesis, but this approach is not possible in non-gene transformable stains. Thus, enhancing PHB level by chemical treatment is desirable because it might be applied to many cyanobacterial species. This study screened 19 compounds previously reported to affect microbial metabolism for their effect on PHB levels in cyanobacterium Synechocystis sp. PCC6803. The exposure to cell membrane interfering agent 2-phenylethanol (2-PE) can significantly increase PHB from 7% up to 33% when combined with the nitrogen-depleted medium. The result showed that the 2-PE-treated cells reduced cellular protein content and down-regulated gene expression in photosynthesis and protein synthesis. In addition, the metabolomic analysis suggested that the levels of metabolite substrates in PHB synthesis (acetyl-CoA, acetoacetyl-CoA, 3-hydroxybutyryl-CoA, and NADPH) were elevated by 1.6–2.7-fold. The non-gene transformable Anabaena sp. treated with 2-PE can increase PHB level from 0.4 to 6.6% (w/w DW). The chemicals of this study might be applied in other cyanobacteria PHB producers.

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Abstract 1380

Binding based proteomic profiling (BBPP) of Fatty acid amide binding protein in *Drosophila melanogaster*

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The role of fatty acid amides (FAAs) as cell signaling lipid has been corroborated by the discovery and extensive works on endogenous FAAs such as anandamide and oleamide. However, studies have been limited to a select few FAAs and much of this class of compounds has not been explored. To elucidate the roles of some of the FAAs, we have employed a two-fold strategy involving i) synthesis and characterization of FAA-targeted BBPP probe with photoreactive diazirine group and a clickable alkyne handle, ii) its use in labelling, identification, and validation of FAA binding proteins in *Drosophila melanogaster*. The diazirine group upon UV irradiation induces the formation of carbene that forms a covalent bond between the probe and the protein to which it is bound, thus allowing selective labelling of the proteome. Further, we utilized the terminal alkyne to perform click reaction to append rhodamine azide for fluorescent imaging, or biotin azide for Western blotting to verify proteome labelling. Biotin tagged proteomes were enriched by subjecting to avidin columns followed by their subsequent identification by high resolution LC-MS/MS. To sum up, BBPP offers a flexible strategy whereby a whole array of FAA analogues can be generated and employed to understand their respective biological roles.

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Abstract 1384

Transposon mobility in serial isolates of *Cryptococcus neoformans* from patients with recurrent cryptococcal meningitis

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Cryptococcus are environmental fungi that cause invasive diseases primarily in populations with weakened immune systems. Inhaled into the lungs, Cryptococcus can persist for months to years and even decades in patients with recurrent cryptococcal meningitis. Therefore, understanding the types of genomic adaptations that occur during infection to enable persistent disease is critical in developing effective treatment strategies. We previously reported transposable element (TE) mobility as a significant driver of mutations in *Cryptococcus deneoformans* during murine infection (Gusa and Williams et al., PNAS, 2020). TE mobility was elevated in response to heat stress *in vitro*, contributing to an increased rate of antifungal drug resistance at 37°C (human body temperature). The objective of this study was 1) to assess whether TEs mobilize in Cryptococcus during human infection and 2) to determine whether heat stress increases the TE mutation rate in *Cryptococcus neoformans*, a major disease-causing species. To perform these studies, we obtained serial clinical isolates of *C. neoformans* from patients with recurrent cryptococcal meningitis. These isolates were previously evaluated in studies that utilized short-read whole-genome sequencing to identify genetic changes between incident and relapse infections (Chen et al., mBio, 2017). A small number of genetic differences were identified comparing patient isolates obtained months apart, including base substitutions, minor insertions/deletions and copy number changes. Importantly, several virulence-related phenotypes observed in the relapse isolates could not be explained by the genotypic changes identified. Because TE movements are often masked in whole-genome comparison studies that utilize short-read sequencing, we sought to uncover TE changes that might have been missed. First, we applied an FRR1 "transposon trap" assay to screen for TE mobility among the clinical isolates. We newly identified an LTR retrotransposon (Tcn3) and a DNA transposon (CNIRT6) as mobile in *C. neoformans* under conditions of heat stress. Comparing the rate of rapamycin+FK506 drug resistance at 30° vs 37° in clinical isolates with demonstrated CNIRT6 mobility, we found a significant increase in drug resistance at elevated temperature. Nearly all drug-resistant mutants obtained at 37° were due to CNIRT6 insertions. Next, we probed genomic DNA from the serial isolates for specific TE sequences. Surprisingly, we detected TE movements/changes in about half of the patient cases screened. Changes in the hybridization pattern for Tcn3, Tcn6 and CNIRT6 were detected among serial isolates from patients from different geographic regions, including South Africa (Chen et al. study) and the United States (Ormerod et al., G3, 2013). Our results suggest that TEs mobilize in

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Cryptococcus during human infection, and that heat stress at human body temperature can significantly increase the TE mutation rate in *C. neoformans*. It is therefore possible that TE mutations in the genomes of cryptococci during infection contribute to microevolution and disease persistence in the host. Long-read whole-genome sequence analyses of the clinical isolates are in progress to map the locations of TE movements in relapse isolates to determine their contributions to phenotypic changes and fitness.

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Abstract 1420

Chemoproteomic Profiling Surveys the Degradable Proteome

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Small molecules that induce protein degradation through ligase-mediated ubiquitination have shown considerable promise as a new pharmacological modality. We and others have demonstrated that efficacious degradation of kinases HDACs and other targets can be achieved *in vitro* and *in vivo*, however, many targets remain recalcitrant to degradation. In this work we took advantage of two library design approaches to build family-centric degrader libraries targeted towards protein kinases and Zinc-dependent HDACs. These libraries, which included 91 kinase degraders and 52 HDAC degraders, were screened using large-scale unbiased quantitative chemo-proteomics in different cell lineages to identify and map degradation targets. We identified more than 200 kinase-like proteins as degradable, a substantial increase from the 57 kinases previously reported as degradable in the literature and 9 HDACs as degradable. Having established tractable targets from the accumulated experimental data, we then evaluated the contributions that chemical and cellular variables have on TPD efficacy and selectivity and found that factors such as ligase recruited, linker length and chemistry, cellular target occupancy and ternary complex formation play a surprisingly inconsistent role in degradation success. We demonstrate that the accumulation of large unbiased datasets such as this can serve as a rich source of small molecule tools for studying biology of different proteins, can enable mapping of tractable and recalcitrant protein targets and can provide chemical lead molecules for many new target proteins.

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Abstract 1460

Identifying GABA de novo synthesis route in cancer cells

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Gamma-aminobutyric acid (GABA) is mostly known as an inhibitory neurotransmitter in the mammalian central nervous system, but it also functions in peripheral tissues to regulate cell proliferation, differentiation, and migration. In order to better understand the function and signaling of GABA in cancer cells, we investigated the biosynthesis route of GABA. Using 13C and 2H tracers, we demonstrated that, unlike GABAergic neurons that utilize glutamate decarboxylases (GADs) for GABA synthesis, cancer cells do not rely on glutamate or glutamine for GABA production. Instead, cancer cells prefer the arginine-ornithine-putrescine pathway for GABA de novo synthesis. Ornithine, which is non-proteogenic and is often omitted from cell culture media, is actually the most preferred substrate for GABA synthesis. We found that fetal bovine serum (FBS) used in cell culture typically contains high arginase activity, and it keeps breaking down arginine to make ornithine for cell metabolism. When culturing cells with heat-inactivated FBS that is free of arginase activity, we used 2H-ornithine and 13C-arginine tracers to show that >90% GABA was made from 2H-ornithine. Taken together, our results demonstrate that cancer cells rely on extracellular ornithine to support GABA de novo synthesis.

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Abstract 1476

1H NMR Metabolomic Multivariate Analysis of Rat Plasma Reveals the Correlation of HIV-1 Genotype with Age and Substance Use

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The noninfectious HIV-1 Transgenic (HIV-1Tg) rat model provides an ideal tool for studying the effects of substances of abuse on HIV-1 infected individuals receiving highly active antiretroviral therapy (HAART). The animal model has been widely used in neurological studies as it represents the constant expression of viral proteins, such as Tat and gp120, that could lead to neurological impairments. However, very little information is available at the metabolic level on how HIV-1Tg rats differ from their Fisher 344 (F344) control animals, and what effects the constant viral protein expression and neuroinflammation can exert on the metabolism of these animals in regards to the age of the animals and the treatment of substances of abuse. In this study, we collected plasma samples from both F344 and HIV-1Tg animals at various ages with and without treatment with methamphetamine. One-dimensional 1H NMR spectroscopy, coupled with principal component analysis (PCA), was applied to investigate the differential metabolic profiling in the plasma samples. Our results indicated that the metabolic profile changes significantly between F344 and HIV-1Tg rats upon aging, suggesting aging may play an important role in the progression of HIV-1-associated neurodegeneration. This study could potentially provide biomarkers and metabolic fingerprints associated with aging, drug abuse, and HIV-1 infection.

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Abstract 1490

Re-evaluation of AAV vector concentrations for knock-in mouse production with genome editing

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Our laboratory has been working on introducing genetic modification technology to construct mouse model libraries for rare/intractable diseases in humans (https://animal.nibiohn.go.jp/research/e_rare-disease-model-library.html) for studying the molecular mechanisms of genes in such diseases. As part of these efforts, we re-evaluated a knock-in mouse production method using a combination of a CRISPR/Cas9 system and AAV vector, established by a group of Prof. Hiromitsu Nakauchi at the institute of medical science, the university of Tokyo (Mizuno et al., iScience, 9:286–297, 2018). The AAV vector in the present study was a single-stranded (ss) AAV6 vector containing the CAG promoter, the EGFP cDNA, the SV40 polyA signal, and a Rosa26 homology arm of 700 bp each on the left and right sides (Vector Builder, Inc.). The concentration of the viral stock solution was 2.87 × 1011 GC/mL. The Cas9 protein and guide RNA (CGCCCATCTTCTAGAAAGAC/TGG) complex (IDT Inc.) was introduced by electroporation into C57BL/6N pronuclear stage embryos obtained by *in vitro* fertilization. Then the embryos were co-cultured with AAV vectors overnight. Some embryos were transferred to uterine foster mothers at the 2-cell stage, and some were cultured *in vitro* to become blastocysts for fluorescence observation. First, at the virus concentration according to the original method (10,000-fold dilution, 2.87 × 107 GC/mL), 48 pups (40%) were obtained from 120 2-cell stage embryos, but no pups fluoresced. Next, 12 blastocysts were obtained from 19 2-cell stage embryos co-cultured with AAV at 1,000-fold dilution (2.87 × 108 GC/mL), and 14 blastocysts from 20 2-cell stage embryos co-cultured with AAV at 100-fold dilution (2.87 × 109 GC/mL), but none of them emitted fluorescence. Finally, 120 2-cell stage embryos treated with a 20-fold dilution (1.435 × 1010 GC/mL) of AAV were transferred to foster mothers, and 17 (14%) pups were born. Five (29%) of the pups showed fluorescence. These results indicate that the AAV vector used in the present study required a higher concentration (~1010 GC/mL) than the original method. Other reports (Yoon et al., Nat. Commun. 9:412, 2018) have also used higher concentrations of vectors. Thus, it is necessary to consider the optimal concentration of the AAV vector to be used in practice to create knock-in mice.

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Abstract 1567

Proteolysis pattern of plant seed proteins; an insight from the endogenous peptidome of germinated and ungerminated chickpeas using LC-MS/MS

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Introduction Proteolysis is a physiological process of protein breakdown into smaller peptides or amino acids with the aid of enzymes. Proteolysis serves many processes such as providing amino acids for new protein synthesis, breakdown of food proteins to provide nutrients, and controlling other physiological and cellular processes. Plant seed proteins, especially legumes are important sources of nutrients, providing an alternative to animal proteins which may have deleterious effects on human health and the environment. However, legumes are less completely digested than dairy or animal muscle proteins. It is known that germination increases protein digestibility of these seeds. **Objective** The changes in protein or peptide composition and the pattern of protein hydrolysis which contributes to the increased digestibility upon germination, is not properly understood. The objective of present study was to characterize changes in the peptidome of germinated and ungerminated chickpeas, to gain insights into and changes in active proteolysis preferences, as indicated by changes in motifs at cleavage termini during germination. **Methods** We designed a protocol to extract peptides <10 kd from 2 different strains of chickpea at 4 different time points of germination. Filtered peptides were prepared for LC-MS/MS and characterised using mass spectrometry, with and without trypsinisation. MS/MS spectra were analyzed using MaxQuant software. Data were visualised using R and Peptigram software. **Results** A clear distinction was seen between the termini of peptides of seeds during the course of germination. Alanine, glutamine and glutamate were preferred at the P1 site (amino acid immediately before the cleavage point) in soaked chickpeas, whereas lysine and arginine dominated the P1 position in germinated samples. Principal component analysis indicated that multiple proteases are acting with different substrate preferences. Tiling visualizations of the peptidome for individual proteins indicated the likely activity of exopeptidases. Proteins present in both soaked and germinated seeds are mainly storage proteins and heat shock proteins, whereas proteins specific to germinated samples are enzymes related to the synthesis of new proteins and to the promotion of metabolic activities in growing seedling. **Conclusion** We find that seed proteins are differentially cleaved before and after germination. We identify the preferences of amino acids at different sites of cleavage. These proteolytic preferences have not been previously documented. A better understanding of this natural, large-scale shift in the proteolysis of seed proteins on germination may increase our understanding of how to improve the digestibility of plant proteins.

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Abstract 1592

Identification of Mitochondrially encoded gene variants associated with the pathogenesis of Venous thromboembolism (VTE)

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Background: Mitochondria plays multi-dimensional role in pathophysiology of various diseases. Oxygen disturbance during hypoxic conditions changes the working dynamics of mitochondrial genes. Polymorphisms or variants in several mitochondrially encoded genes like MTRNR1, MTND4, MTND3 have been reportedly associated with different cardiovascular diseases. Till date, no study is available on role of mitochondrially encoded gene variants in venous thromboembolism (VTE) susceptibility, especially under hypoxic condition.

Methodology: In the present study, whole exome sequencing (WES) was employed to identify candidate gene variants in mitochondrially encoded genes. Indian Army volunteers were divided into three study groups, (i) high altitude-VTE patients (n = 6), (ii) sea level- VTE patients (n = 15) and (iii) age-sex matched healthy subjects (n = 19). DNA was isolated from whole blood and WES was performed on Illumina HiSeq platform. Bioinformatic analysis was done to find out the relative presence or absence of variants across the three study groups.

Results: WES data enlisted a total of 180 mitochondrially encoded genes variants in three study groups (high altitude VTE, n = 49, Sea level VTE, n = 69 and healthy control individuals, n = 62). Data was processed further to remove duplicates and missing information, which resulted in 91 mitochondrial variants, for further analysis. These variants were subjected to statistical analysis (Fischer exact test) to identify statistically significant polymorphisms. Nine variants showed statistically significant difference ($p < 0.05$) amongst all study groups. MT-ND4 (rs2853495), MT-RNR1 (rs2853518), rs2853513, rs71900648 and rs3087742 gene variants showed significant appearance in SL-VTE group, whereas MT-ND4 (rs2853497), MT-CYB (rs28357684) and MT-CO3 (rs2248727) were significantly present in HA-VTE group, compared to healthy controls. Interestingly, MT-CYB (rs3135031) was common to both patient groups. Variants rs3135031 and rs2853497 are non-synonymous in nature. MITOMAP database showed that four of these variants have been listed under top 40 high frequency ancestral variants.

Conclusion: Present study identifies specific mitochondrial gene variants associated with VTE susceptibility for the first time. These variants could be validated further and may prove to be crucial biomarkers in the prediction of VTE susceptibility, both at sea level and at high altitude. Our preliminary findings may pave way for better management of unprovoked VTE.

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Abstract 1678

Defining nanoscale genetic interactions of bacteria with engineered nanoparticles

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Engineered nanoparticles have distinct physicochemical properties which enable their utilization in a variety of sectors from Biomedicine to Environmental remediation. As a result, the production of engineered nanoparticles has considerably risen over the years. Engineered nanoparticles interact with biological systems potentially changing the behavior of these systems by inducing specific physiological and metabolic modifications within the exposed organisms; furthermore, these responses to engineered nanoparticles may also differ from one organism to another. While a great deal of work has been done to identify lethal/antimicrobial nanomaterials to control pathogenic microbial biofilm formation, little work has been done to study non-lethal impact of nanomaterials of microbes. Moreover, the genetic impact of this cell-nanoparticle interaction is not well understood. We have defined the transcriptional genetic response of the gram-negative bacterium *Escherichia coli* (*E. coli*) to three different engineered silica, gold, and polystyrene nanoparticles. Screening an *E. coli* reporter gene library that covers 70% of the *E. coli* genome, we have identified eights genes that are upregulated in response to nanoparticle exposure and possibly represent a common nanoparticle response mechanism. These eight genes have been verified using qRTPCR and include previously identified stress response genes (rssB, evgA, sodC,) genes encoding transports (yhdY, yhhT) and several genes with unknown function (glcC, vacJ/MlaA, cysQ). The gene ontology of the eight genes shows that metabolic pathways, signal transduction pathways, oxidative stress and protein transport systems are significantly affected in response to the three nanoparticle exposures. These results demonstrate that there is specific common response of *E. coli* to round-shaped metalloid, metal, and polymeric nanoparticles. Overall, this research will provide a better understanding of bacteria stress response as well as bacterial resistance to nanoparticle-based antibiotics and identify potential new targets for drugs given that the bacteria-nanoparticle interactions have crucial implications in public health and the environment.

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Abstract 1762

Isolation, Characterization, and Genomic Comparison of Novel Bacteriophage Ennea to CR Cluster Bacteriophages Isolated from Tropical Soils in Puerto Rico

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The bacteriophage population represents the greatest diversity in the biosphere, with an estimate of 1031 viruses. Very little is known about these organisms and how they developed reproduction mechanisms through bacterial hosts. Still, they have a great potential to be the next medical milestone for fighting bacterial infections through phage therapy which represents the only current alternative to fight the increasing number of antibiotic-resistant bacteria. By 2050, antibiotic-resistant bacteria are expected to kill 10 million people, according to a report by the United Nations Organization. Ennea is a novel Gordonia terrae bacteriophage isolated from the tropical soils of Puerto Rico. The bacteriophage was enriched from a soil sample and purified at least twice by streaking on a bacterial host lawn before extracting and sequencing its DNA. Enneás clear plaques indicate that it uses a lytic cycle. It is classified as a member of cluster CR of Gordonia terrae phages. A genomic comparison of six CR phages from Puerto Rico shows similar sizes, synteny, number of open reading frames (ORFs), and GC content. As expected, Ennea shows a phylogenetic relationship with all the CR cluster phages from Puerto Rico but is particularly closer to those in subclusters CR1 and CR2 and separates itself from CR4, as evaluated through the Neighbor-Joining method. All the evaluated criteria indicate that these bacteriophages evolved from a common ancestor, which can be pivotal in studying phage evolution in areas with diverse ecosystems, such as Puerto Rico. The island´s diversity includes salt marshes, mangroves, dry and rain forests, and a karst region, among others. Isolation and characterization of these viruses help us obtain a greater understanding of their genetic composition and evolution.

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Abstract 1795

Aberrant expression and somatic mutations in the MUC3A gene of South Africans with Esophageal Squamous Cell Carcinoma

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Estimates for 2017 indicate that 20% of cancers globally are GIT cancers, with oesophageal cancer being the 8th most common cancer. Esophageal squamous cell carcinoma (ESCC) occurs in the upper to mid esophagus and is present at high incidence in developing countries including South Africa. Patients often present with late-stage disease with poor prognosis. The objective of this study was to investigate somatic mutations present in South African ESCC patients and to determine their association and contributions to tumour development and/or progression. Tumour and paired blood DNA were obtained from 35 ESCC patients and subjected to whole genome sequencing (WGS), while 15 pairs were further subjected to RNA sequencing (RNA-seq). Bioinformatics analysis pipelines were designed to investigate the somatic mutations present in the cohort through WGS analysis, RNA-seq analysis and functional enrichment analysis. Immunohistochemical staining was performed on 13 tumour samples to investigate and correlate protein levels. Ethical approval for the study was obtained from the University of Cape Town's Human Research Ethics Committee in compliance with the declaration of Helsinki principles. Very significantly, it was determined that 86% of the patient cohort presented with somatic mutations in the MUC3A gene, an interesting observation as no such previous association with ESCC has been recorded. MUC3A is a membrane-bound glycoprotein component of mucous gels and its aberrant expression has been correlated with invasion and metastasis in a variety of other cancers. Analysis of RNA-seq data showed a 4.6 log2 fold increase in MUC3A expression in the tumour samples of these ESCC patients, with a P-adjusted value of 7.05e-06, suggesting highly significant differential gene expression. Functional enrichment analysis further showed that MUC3A was significantly associated with one of the top 5 gene ontologies (extracellular matrix structural constituent) for molecular function ontology class together with a number of collagen (COL) and MMP genes known to play a role in oncogenic progression and membrane stiffness. GSEA and KEGG analysis indicated predominantly chemokine/cytokine pro-inflammatory enriched pathways with some indication of viral influence. Immunohistochemical staining showed that for samples in which MUC3A mutations were identified in the WGS data analysis, no detectable levels of protein were observed, suggesting that the production of a non-functional truncated protein may lead to the upregulation of MUC3A expression that could play a role in downstream pro-oncogenic signalling. Taken together, these data suggest that MUC3A could be a gene of interest in these ESCC patients and may play a significant role in tumour development and/or progression.

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ASBMB

Abstract 1797

Identification and Characterization of a Prostaglandin Synthase From the Genome of *Mycobacterium smegmatis*

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Prostaglandin synthase catalyzes the conversion of arachidonic acid to prostaglandin H2 in two steps: the addition of two oxygen molecules to form a cyclic oxygen species and a hydroperoxide, followed by reduction of the hydroperoxide to an alcohol. Prostaglandin synthases have been identified in most higher organisms, but no bacterial enzyme has been found that catalyzes both oxidation and reduction reactions. We have expressed a putative prostaglandin synthase identified in the genome of *Mycobacterium smegmatis* and are characterizing its enzymatic reactions to determine if it can catalyze both lipid oxidation and reduction reactions. The Mycobacterium protein was expressed using three different expression vectors yielding proteins with an N-terminal (his)6 tag (HisLicMyco), an N-terminal SUMO partner (SUMO-Myco), and an N-terminal thioredoxin partner (Trxt-Myco). The SUMO-Myco construct was not enzymatically active, but the other two constructs possess dioxygenase activity, measured using an Instech oxygen monitoring system. Oleic, linoleic, and α-linolenic acid were all substrates for the enzyme with Km values ranging between 10 and 263 mM. All three constructs demonstrate peroxidase activity using hydrogen peroxide as a substrate. Using a spectrophotometric assay, HisLicMyco and Trxt-Myco were shown to consume both oxygen and electrons in the presence of a lipid substrate. Finally, liquid chromatography/high resolution mass spectrometry confirmed the generation of lipid hydroperoxides that were reduced to alcohols when the electron donor epinephrine was added. The data indicates that the Mycobacterium enzyme catalyzes both dioxygenase and peroxidase reactions and potentially functions as a prostaglandin synthase. If confirmed, this represents the first identification of a bacterial prostaglandin synthase. *Mycobacterium smegmatis* is pathogenic, so an understanding of this enzymatic activity can give some insight into the potential products expelled from the bacteria during infection, which can be related to a more serious bacterium in the same genus, *Mycobacterium tuberculosis*.

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Abstract 1807

Expression and Characterization of a Heme-Dioxygenase from the *Cyanobacterium cyanothece*

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Prostaglandin H2 synthase (PGHS) catalyzes the conversion of arachidonic acid to prostaglandin H2, which is subsequently converted into prostanoids. To date, no bacteria homolog to mammalian PGHS has been identified, but several potential PGHS enzymes have been identified in bacterial genomes. In this study, the protein product of a putative PGHS from Cyanothece was expressed and characterized to determine if it has prostaglandin synthase activity. The protein was expressed as an N-terminal SUMO fusion protein, with the SUMO partner successfully removed using SUMO hydrolase. Using an oxygen monitoring system, dioxygenase activity of the Cyanothece enzyme was measured with the lipid substrates linoleic acid, oleic acid, and a-linolenic acid. The kcat/Km values for a-Linolenic, oleic, and linoleic acids were 0.18 s-1 mM-1, 0.12 s-1 mM − 1, and 0.093 s-1 mM − 1, respectively, indicating a lack of preference by the enzyme for C18 lipid substrates. Product analysis from reactions with linoleic acid (C18H32O2) using liquid chromatography/high resolution mass spectrometry demonstrated the presence of a C18H32O4 product which confirms dioxygenase activity. The enzyme does not exhibit peroxidase activity using hydrogen peroxide as the substrate. The Cyanothece dioxygenase will be compared to heme dioxygenases previously identified in tobacco and Arabidopsis to help us to better understand the structure and function of the bacterial enzyme.

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Abstract 1817

Use of mass spectrometry-based proteomics to study post-translational modifications of the mitochondrial transcription machinery

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Mitochondria are unique sub-cellular compartments that contain their own genome which contains the genetic code for 13 protein subunits required for the synthesis of ATP. Currently, the regulation of mitochondrial gene transcription in response to changing energetic needs is not well understood. We hypothesize that protein post-translational modifications (PTMs) play a role in nutrient sensing and the control of mitochondrial transcription, similar to some mechanisms that control nuclear gene expression. We used liquid chromatography tandem mass spectrometry (LC-MS/MS) to identify PTMs on the mitochondrial protein expression machinery, including the mitochondrial RNA polymerase protein (POLRMT) and the ribosomal protein L12 (MRPL12). POLRMT and MRPL12 were overexpressed in mammalian cells and immunoprecipitated. Immunoprecipitated proteins were subject to trypsin digestion and the resulting peptides were analyzed by LC-MS/MS. Spectral library searching was used to identify peptides and PTMs. We obtained 41% sequence coverage for MRPL12 and 20% for POLRMT. We identified three distinct PTM sites on MRPL12 and nine on POLRMT, including acetylation and phosphorylation sites. These PTM sites are being further characterized using *in vitro* and cellular studies to develop a better understanding of the roles of PTMs in mitochondrial transcription and translation regulation.

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Abstract 1832

Regulation of inflammatory Gene expression in Senescence

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Mammalian cells express a set of inflammatory genes when they are induced into senescence, although the set of induced genes appears to vary depending on the cell type and inducing stimulus. This inflammatory phenotype is thought to have beneficial effects in some processes such as wound healing. The expression of inflammatory factors may also recruit immune cells that could eliminate the senescent cells. However, the observed accumulation of senescent cells with ageing suggests that the immune system is ineffective at eliminating senescent cells during ageing. This accumulation of senescent cells creates a chronic low-level inflammation during ageing that can contribute to age-related diseases. The project involves characterising the diversity of inflammatory gene expression in primary human fibroblasts. Most published transcriptomic studies of human fibroblasts involve a small number of cell lines derived from embryonic lung tissue (WI38, IMR90). The available data suggest that these cells express inflammatory genes when induced into senescence. While characterising primary human mammary fibroblasts, we were surprised to see that these cells expressed fewer inflammatory genes when induced into senescence compared to WI-38 cells. This result indicated that expression of an inflammatory SASP may not be as frequent as the current literature would suggest. We performed RNA-seq analyses of primary human fibroblasts from diverse tissues before and after the induction into senescence by ionising irradiation in order to explore the diversity of their expression of inflammatory genes. The primary fibroblasts were derived from different anatomic sites (abdomen, breast, lung, scalp, trachea), and from several individuals of different ages and sexes. Many of these primary fibroblasts showed lower SASP expression patterns compared to WI38. The Comparative transcriptomic analyses of fibroblasts that are SASP-competent and incompetent identified candidate pathways that are required for SASP expression such as IL1A which was lowly expressed in all the primary human fibroblasts compared to WI38. ATAC-seq analyses will allow comparisons of open chromatin features amongst the different fibroblast types that may pinpoint key regulatory regions that distinguish them.

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Abstract 1847

Identifying the enzyme responsible for initiating aerobic acetylene metabolism in *Rhodococcus rhodochrous* ATCC 33258

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Acetylene (C2H2) forms in anoxic methane-rich atmospheres such as those found on early Earth. And may represent an early carbon and energy source for bacteria. Anaerobic acetylene-utilizing strains such as Pelobacter ferment acetylene and can grow on anthropogenic sources of this gas. They are also known to play a role in the degradation of environmental contaminants. The first step of anaerobic acetylene metabolism is catalyzed by acetylene hydratase (AH) which generates acetaldehyde. The crystal structure of this enzyme has been determined and the roles of metal centers including tungsten and Fe-S centers has been studied spectroscopically. *Rhodococcus rhodochrous* ATCC 33258 is an example of an aerobic acetylene-utilizing bacterium and unlike Pelobacter, the enzyme responsible for initiating acetylene metabolism in this bacterium has not been characterized. In this study, we have used genome-enabled analyses to investigate the enzymology and physiology of this bacterium. The genome of *R. rhodochrous* contains a candidate AH that is structurally similar to the enzyme found in Pelobacter but likely utilizes molybdenum as opposed to tungsten; and also lacks a tungsten import system. Acetylene uptake studies using gas-chromatography on *R. rhodochrous* cells grown on diverse carbon compounds suggests constitutive AH expression. Although, cells grown on 1-propanol exhibit unusually high rates of C2H2 uptake. Preliminary shotgun proteomic analysis confirmed the expression of AH in acetylene-grown cells, along with several currently unidentified proteins. Future studies will focus on analyzing pathways associated with acetylene metabolism by comparing protein expression profiles with cells grown on different carbon compounds.

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Abstract 1867

Unique Expansion of PARP Family Proteins in the *Fusarium oxysporum* species complex

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From devastating agricultural crops to instigating keratitis public health crises, the *Fusarium oxysporum* species complex (FOSC) contains a group of dynamic fungal pathogens with a strong host specificity, determined by strain specific accessory chromosomes. Understanding the regulatory mechanisms that govern pathogenicity is essential for the development of effective agricultural and medical methods to control *F. oxysporum* infections. The poly-ADP ribose polymerase (PARP) protein family participates in numerous regulatory cellular functions, including DNA repair, apoptosis, chromatin remodeling, and cell cycle regulation by synthesizing long chains of ADP-ribose molecules in a process called PARylation. Comparative genomic analysis revealed that the PARP family is uniquely expanded in FOSC, ranging from three to twenty copies across strains. The expansion copies are primarily located in accessory chromosomes. Utilizing four strains of *F. oxysporum*, we created a comparative system to investigate how PARP copy number affects DNA damage tolerance and virulence. We found that PARP copy number is positively correlated with survival rates in the presence of DNA damage agents, specifically methyl methanesulfonate (MMS) and H2O2, and with basal cellular PARylation levels. Knocking out the Parp1 gene, which is the primary DNA repair PARP, in all four strains, significantly reduced infection severity in *Arabidopsis thaliana* and tomato plants, and increased sensitivity to human macrophages. These results suggest that the PARP family in *F. oxysporum* plays a role in the response to DNA damage but is also involved in pathogen-host interactions. We believe that these findings open future possibilities for further investigating the precise mechanisms of this host-microbe interaction.

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Abstract 1909

Enrichment and Analysis of Head and Neck Cancer Mucin-Domain Glycoproteins Reveals the Presence of Tumor-Derived Immunoglobulin A

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Mucin-domain glycoproteins expressed on cancer cell surfaces play central roles in cell adhesion, cancer progression, cell renewal, and immune evasion. These glycoproteins are characterized by the presence of serine, threonine, and proline-rich O-glycosylated regions. Despite abundant evidence that mucin-domain glycoproteins are critical to the pathobiology of head and neck squamous cell carcinoma (HNSCC), our knowledge of the composition of that mucinome is grossly incomplete. The objectives of this study are to: (a) test a strategy for the enrichment of HNSCC mucin-domain glycoproteins, and (b) identify mucin-domain glycoproteins in HNSCC with a focus on glycoforms that are uniquely expressed in aggressive tumor cells. To this end, we enriched mucin-domain glycoproteins in HNSCC cell line lysates utilizing the catalytically inactive recombinant StcE (secreted protease of C1 esterase inhibitor), followed by their characterization using SDS-PAGE, in-gel digestion, nLC-MS/MS, and enrichment analyses. Among the identified mucin-domain glycoproteins common to all assessed cell lines are agrin (AGRN), podocalyxin (PODXL), CD44, and syndecan-1 (SDC1). Several proteins, including immunoglobulin A, were enriched uniquely from aggressive tumor cell lines. One explanation for this finding could be that a unique glycosylated form of the IgA1 heavy chain constant region is present in aggressive HNSCC cell lines. Intriguingly, it has previously been reported that sialylated tumor-derived immunoglobulins, expressed by epithelial tumor cells rather than immune cells, play a glycosylation-dependent role in tumor immunosuppression. These findings represent the first attempt to identify mucin-domain glycoproteins in HNSCC in an unbiased manner, paving the way for a more comprehensive characterization of the mucinome components that mediate aggressive tumor cell phenotypes.

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Abstract 1920

Liquid Chromatography/ High-Resolution Mass Spectrometric Analysis of Reaction Products From a Putative Bacterial Cyclooxygenase

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Teresa Rotolo, Barry Selinsky

The Selinsky lab has been investigating the protein product of an open reading frame in the genome of *Mycobacterium smegmatis* which is annotated as a prostaglandin synthase (Myco-PGHS) based upon predicted structural similarity to the mammalian enzyme. The lab has expressed Myco-PGHS and discovered that it exhibits both dioxygenase and peroxidase activities with lipid substrates, suggesting that the enzyme is a prostaglandin synthase. I have conducted structural analyses of Myco-PGHS reaction products from lipid substrates using high-performance liquid chromatography/mass spectrometry (LC/MS). Myco-PGHS was reacted with linoleic acid, linolenic acid, arachidonic acid, or oleic acid in oxygenated buffer either with or without the electron donor epinephrine. Reactions are quenched with acid or acidic SnCl2. Products were collected by solid phase extraction and resuspended in methanol for LC/MS analysis in negative ion mode. The analysis shows the generation of O6 and O4 containing compounds in the absence of reducing agents, indicating that the enzyme adds one or two oxygen molecules to the lipid substrates. With linoleic acid, molecular mass of 280.4 g/mol, the addition of oxygen molecules generated products with molecular masses of 312.2 and 344.2 g/mol, indicating the addition of one and two oxygen molecules, respectively. When the products are reduced with SnCl2, MS reveals products with molecular masses of 328.2 and 296.2 g/mol indicating reduction to O5 and O3 products. When epinephrine is included in the reaction, the 312.2 and 344.2 g/mol products do not appear in the mass spectra, but no other unique products are identified. These findings suggest Myco-PGHS exhibits cyclooxygenase activity. Further experiments will be performed to identify the products from epinephrine reduction and to determine the structures of the products being formed. These findings are significant because Myco-PGHS would be the first bacterial cyclooxygenase and may represent the evolutionary precursor to mammalian prostaglandin synthases.

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Abstract 2021

Metallomics profiling of specific tissues from four developmental ages of Transmembrane 163 (Tmem163) knockout mouse

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Natalie Shink

Human TMEM163 is a protein that has been reported to bind zinc, copper, and nickel. Our laboratory recently showed that TMEM163 transports zinc into intracellular compartments and out of cells, making it a zinc efflux transporter. TMEM163 has been implicated in several human disorders such as Mucolipidosis type IV, Hypomyelination Leukodystrophy, Parkinson's disease and diabetes. We have previously shown that knocking down the expression of human TMEM163 in cultured cells creates an imbalance of intracellular zinc levels, suggesting that it is likely critical for zinc homeostasis in various cells and tissues. To further dissect the function of TMEM163, we used an animal model in which its mouse counterpart, Tmem163, has been knocked out. We hypothesized that the loss of mouse Tmem163 protein expression will result in abnormal metabolism of metals, particularly metals that were reported to bind Tmem163 such as copper, nickel, and zinc. We used post-mortem tissues from Tmem163 knockout (KO) and wildtype (WT) mice, consisting of cerebral cortex, cerebellum, lungs, and pancreas, because of prior knowledge that Tmem163 transcripts are highly expressed in these tissues. Using inductively coupled plasma mass spectrometry (ICP-MS), we analyzed the levels of metal isotopes of iron, nickel, copper, zinc, sodium, magnesium, calcium, and manganese from KO and WT tissues of 1-, 3-, 7-, and 16-month-old mice. We also used the Timm Sulphide Silver stain to analyze the localization and distribution of neuronal zinc in the brain of 1-, 3-, and 7-month-old KO and WT mice to corroborate the ICP-MS analysis on cerebral zinc levels. Our ICP-MS results showed a significant elevation of iron, sodium, magnesium, and manganese in 1-month-old brain tissues of Tmem163 KO mice when compared to WT controls. While zinc levels in Tmem163 KO brain tissues were unchanged in 1- and 3-month old developmental ages, there was a significant decrease of zinc in the lungs of 1- and 3-month-old Tmem163 KO mice when compared with WT controls. In both cortex and cerebellum of 7-month-old Tmem613 KO mice, zinc levels were significantly decreased compared to WT controls. Interestingly, iron, copper, zinc, sodium, and magnesium levels were also significantly decreased in brain tissues of 16-month-old Tmem163 KO mice when compared to WT controls, but there was a significant elevation of calcium. Our histochemical results appear to show a relative reduction of Timm staining for hippocampal zinc of 1- and 7-month-old Tmem163 KO mice in comparison to WT mice of the same age. Overall, these observations show that mouse Tmem163 is crucial for metal homeostasis among distinct tissues examined during development. Furthermore, our results suggest that human TMEM163 plays an important role in human health and disease.

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Abstract 2078

Multi-omic analysis of metabolites, proteins, and histone modifications during human macrophage polarization

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Background: Polarization between M1-MΦ and M2-MΦ is intelligently utilized by our immune system to safeguard extrinsic pathogen invasion by killing them with M1-MΦ then apply anti-inflammatory M2-MΦ to repair cytokine-caused tissue damage. However, uncontrolled macrophage polarization contributes to the pathophysiology of metabolic syndromes, infectious and autoimmune diseases, asthma, and cancers. A new concept of the interplay of metabolism and epigenetics has emerged to regulate macrophage polarization. however, the high complexity of metabolites hinders the study of such connection. Though modification enzymes that add modifiers to the chromatin or remove them has been extensively studied for more than two decades, numerous lines of evidence suggested that methyl- and acetyl-pools taken from dietary or generated from cellular metabolism intermediates significantly contribute to chromatin modifications, linking nutrition and central energy metabolism to gene expression plasticity. Both mathematical models and experimental observations revealed that acetylation changes due to metabolic states; shifting from low to high glucose significantly affects protein acetylation level, so does the changes of acetate, hypoxia, or starvation of amino acids. As there is an intimate link between metabolism and histone modifications, we hypothesize that the crosstalk between metabolism and epigenetic modifications determines macrophage polarization states optimized for immune response and inflammation. **Methods:** A novel triomics method was employed to analyze metabolites, histone modifications and protein expression from the same sample preparation of M0-, M1-, and M2-MΦs. Isotope-tracing to monitor methyl transfer from serine to histones and acetyl transfer to histones were carried out to verify the data garnered from non-isotope experiments.

Results: Blocked TCA cycle that is still be maintained by replenishing OAA through glutamate/aspartate metabolism, upregulated de nova synthesis of NAD+ from tryptophan metabolism and increased NAD+ production by oxidation of NADH via the mitochondria respiratory complex I to increase Suituin-type histone deacetylase activity, and accumulated Nα-Acetyl-Aspartate (NAA), -glutamate (NAG), and -ornithine resulting in the trap of acetyl-CoA, contribute together to hypoacetylation of histones measured in M1-MΦ. The blocked one-carbon metabolism results in histone demethylation in M1-MΦ; contrastingly, arginine di-methylation of RG or GR di-amino acid peptides is increased in M1-MΦ. **Discussion and Conclusions:** The triomics data clearly shows the cross talk between metabolism and histone modifications during macrophage polarization. Although differential expression of histone acetyltransferases and deacetylases cannot be completely ruled out, lower acetyl-CoA production from glycolysis and blocked acetyl release from NAA, NAG and NAO, in addition to increased NAD+ synthesis or production may be the major causes for reduction of histone global acetylation in MΦ. Significantly elevated di-methylated arginine-glycine peptides in M1-MΦ suggests that RG/GR-domain containing proteins such as H4-R3 and RelA-R30 are hypermethylated and that arginine methylation by arginine specific methyltransferases overcomes the reduction of SAM due to one-carbon blockage in M1-MΦ. Derailing these metabolic pathways could lead to unique epigenetic landscape of the macrophage that prefers to either pro- or anti-inflammation.

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ASBMB

Abstract 2188

Threonine-503 is Critically Important for Activity in the *Gracilaria vermiculophylla* Prostaglandin Synthase

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Salko Hrnjic, Barry Selinsky

The red algae *Gracilaria vermiculophylla* expresses a prostaglandin synthase (PGHS) capable of converting arachidonic acids into prostaglandin products. In contrast to mammalian prostaglandin synthase, the algal enzyme is not inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs). An active site serine residue in the mammalian enzyme is replaced by a threonine in the algal enzyme (T503), which may explain the lack of NSAID inhibition. We wish to test if mutating T503 will cause the algal PGHS to demonstrate inhibition by NSAIDs. Site directed mutagenesis was performed to mutate T503 to serine (T503S) or alanine (T503A). Function of the T503S and T503A mutant enzymes were evaluated through peroxidase and dioxygenase activity assays. The T503S mutant is completely inactive, while the T503A mutant maintained a minimal amount of peroxidase activity but dioxygenase activity was eliminated. I conclude that T503 must be critically important for the catalytically activity of the Gracilaria PGHS enzyme. Further exploration of other residues that contribute to the enzyme active site is necessary to better understand the enzymatic mechanism of the Gracilaria PGHS relative to its mammalian homolog.

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Abstract 2191

Defining properties of lipid scavenging in cancer

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Scavenged lipids are used to generate membranes for proliferating cells; but, which of the various lipids found in serum are scavenged and their respective consumption rates from the environment are unknown. We have used liquid chromatography mass spectrometry (LCMS) to rigorously and unbiasedly quantify lipid consumption rates of different classes and species of lipids across a panel of cancer cells to generate a preliminary landscape of lipid consumption patterns. Interestingly, despite access to a diverse repertoire of lipids, ranging from neutral lipids and sterols, to phospholipids and free fatty acids, lipid consumption in A549 cancer cells were restricted to free fatty acids and lysophosphatidylcholine molecules, which are phosphatidylcholine molecules that have only a single fatty acyl chain. Understanding lipid consumption patterns and variability in cancer reveals potentially targetable vulnerabilities in lipid metabolism by eventually developing small compounds to inhibit scavenging of particular lipids in the management of cancer.

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Abstract 2192

Design and construction of a synthetic mycobacterium phage genome using transformation-associated recombination (TAR) cloning in *Saccharomyces cerevisiae*

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Debora Edouard, Makayla Martorana, Riana Pozsgai, Jennifer Roecklein-Canfield

The rise of antibiotic resistance in pathogenic bacteria is driving a renewed interest in bacteriophages. These naturally occurring antimicrobial agents can potentially be exploited as novel therapeutic agents. Existing limitations such as bacterial resistance and narrow host range; however, are problematic. The advent of new techniques in genome science and synthetic biology have provided mechanisms to build unique phage genomes that confer new functions that may overcome these difficulties. Building upon methods developed in the Build-a-Genome course at Johns Hopkins University and the Synthetic Yeast Project (www.syntheticyeast.org), we present the construction of a synthetic genome of the mycobacterium phage Giles. We adapted the novel assembly line approach introduced by Oldfield et al in 2017 and set out to construct the complete genome from 12 overlapping DNA fragments of the phage using the yeast based transformation-associated recombination (TAR) system. Individual mini-chunks of DNA are incorporated via polymerase chain reaction (PCR) with a TAR cloning vector using specifically designed primers. The DNA fragments are then assembled into the full length phage genome inside yeast. Genetic modifications can be made by attempting to insert novel DNA sequences into one or more fragments. The final objective of this project is to transform the synthetic phage into bacterial cells and measure its infectivity. The results of this project will show a successfully engineered and infectious clone of phage Giles. The significance of which will be to demonstrate the optimization of novel methods to construct a synthetic phage genome.

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Abstract 2206

Vaccinia and Venezuelan Equine Encephalitis Virus-Infected Human Dendritic Cells: Transcriptomic Analysis of Host Responses

Stacy-ann Miller, *Walter Reed Army Inst of Research*

Aarti Gautam, Ruoting Yang, SBC FCC, Sofi Ibrahim, Kevin Swift, Marti Jett, Rasha Hammamieh

Understanding host cell response to viral infection could lead to the identification of molecular targets that can be used for the development of diagnostics and therapeutics. In this study, we investigated human dendritic cell (DC) response to infections with Vaccinia (VAC), a highly immunogenic poxvirus and Venezuelan Equine Encephalitis (VEE), a single-stranded positive strand RNA Alphavirus to present a multi-omics comparative analysis of virus host response in dendritic cells across time. Comparative changes in DC mRNA and miRNA expression resulting from infection by the two viruses at 1-, 8-, and 12-hours post-infection (hpi) were determined. Most changes in mRNA and miRNA expression occurred during 8–12 hpi. Both viruses induced apoptotic and immune response pathways during this period, including FOS, IFNA2 and IFNB1. Expression of genes involved in the proinflammatory, blood circulation and regulation of innate immune response pathways was more pronounced in VEE-infected cells than VAC-infected-cells. VAC-infected cells showed selective expression of genes involved in platelet activation, DNA damage and macromolecule synthesis pathways. Both mRNA and miRNA expression showed stronger VAC separation from uninfected controls at later time-points than VEE. The ability to identify early molecular targets for viral infections could potentially lead to improved pre-symptomatic surveillance and diagnosis. By matching differently expressed genes to specific miRNA changes, we will hopefully gain a better understanding of the temporal regulatory dynamics of host-virus response.

Disclaimer: Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense.

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Abstract 2218

Analyses of metabolites over time in deployed active-duty service members

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Active-duty military exposed to traumatic warzone events are at an increased risk for developing PTSD. Pre-deployment identification of risk/ resilience factors is crucial in developing strategies to prevent PTSD. The current cohort is a prospective longitudinal study designed to accelerate the development of sensitive and specific biological markers to aid in diagnosis of PTSD. We investigated the consequences of combat elicited PTSD based on blood metabolomics. Data were collected prior, three days after a 10-month deployment and 90–180 days post-deployment. Based on the psychological scores, the cohort was divided as: (PTSD (N = 146)), PTSD sub-threshold (N = 171), High resilience group (HRG), (N = 502) and Low resilience group (N = 505)). Both PTSD and sub-threshold group have significantly higher Glycolytic ratio ([Pyruvate + Lactate]/ Citrate) than HRG, while both groups have a significantly lower global arginine bioavailability ratio (arginine/[ornithine + citrulline]). The PTSD group also has lower serotonin and higher glutamate than HRG, which is clinically correlated to depressive and anxiety symptoms in PTSD group. These metabolic differences were not explained by gender, age, body mass index, smoking, or consumption of energy drinks/coffee. These findings have clinical implications for assessing pre-deployment factors predictive of risk and understanding evolution of PTSD.

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Abstract 2244

Genomic and metabolic characterization of a novel Gluconobacter sp. isolated from grapes

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The objective of our study was to isolate, identify, sequence, and characterize a wild bacterial isolate from the Finger Lakes Region of New York, with a focus on bacteria that can do fermentation. We collected samples from orchards and vineyards near the SUNY Geneseo campus, including wild fruits such as apples and grapes. Fruit samples were mixed with tryptic soy broth to form a slurry, plated on tryptic soy agar, and isolated using streak plating. Colonies of interest were viewed via microscopy, and DNA was extracted using a Qiagen Powersoil kit. Sequencing of the 16S region identified our bacterium as a species of Gluconobacter, and whole genome, paired-end Illumina sequencing was carried out by SeqCenter (Pittsburgh, PA). The genome was assembled and annotated using the Galaxy platform. The Gluconobacter sp. genome is approximately 3.5 Mb in size, with 3,623 predicted coding sequences. Genome annotation revealed the presence of a predicted levansucrase enzyme, which forms fructose polymers from sucrose. A comparison to protein sequences of microbes with known levansucrase activity showed that the three amino acids necessary for enzyme activity were conserved. Thus, it is likely that our Gluconobacter strain has a functional levansucrase enzyme. Growth on sucrose-containing media induced the expression of levans, and levans were isolated via centrifugation and ethanol precipitation. Levan production was quantified via spectrophotometry, and estimated to be approximately 3 mg/mL. Current experiments will characterize the types of levans produced by our strain. In addition, we have isolated RNA via bead beating in combination with the TRIzol reagent, followed by purification using an RNA isolation kit. Illumina RNAseq will be carried out on RNA from samples grown on either sucrose or glucose. RNAseq will allow us to gain insight into the transcriptional profile of our Gluconobacter strain, including analysis of levansucrase gene expression changes, and will be used to improve the genome annotation of our strain.

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Abstract 2263

Detection and Quantification of Proteins Using Protein Identification by Short-epitope Mapping (PrISM)

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Introduction: We demonstrate Protein Identification by Short-epitope Mapping (PrISM), which aims to provide comprehensive proteome analysis with broad dynamic range at single-molecule resolution by interrogating immobilized, intact proteins in parallel using multi-affinity probes. Improving the dynamic range and scale for protein analyses will enable a deeper understanding of low abundance proteins in biological samples, which has implications in fields such as metabolism, drug discovery, and cell signaling. In addition, enabling the interrogation of single-molecules will provide a more comprehensive view of protein diversity, such as the proteoforms resulting from post-translational modifications inherent to disease states like cancer and Alzheimer's Disease. The combination of single-molecule sensitivity with comprehensive proteome coverage could also open the door for highly sensitive and specific diagnostics in the future.

Methods: PrISM uses non-traditional multi-affinity probes with high affinity and low specificity that bind to short epitopes present across many proteins. Sample proteins were conjugated to DNA nanoparticles and deposited on a high-density patterned flow cell at optically resolvable locations. Multi-affinity probes were applied to sample proteins over multiple cycles to generate binding patterns for each single-molecule protein, which are translated to protein identifications and quantities using a machine learning analysis. We acquired PrISM data on native biological and control samples using dozens of multi-affinity probes targeting trimer or tetramer sequences.

Results: We report single-molecule deposition of over 1 billion DNA nanoparticle protein complexes on a flow cell. We demonstrate how the PrISM methodology identifies individual protein molecules through iterative probing with our multi-affinity probes. Further, we provide an analytical assessment of the sensitivity and specificity of PrISM and demonstrate the ability to accurately estimate the false identification rate of these proteins using a target-decoy based statistical approach.

Conclusions: Combining single-molecule analysis, intact (non-digested) proteins, and iterative probing, PrISM provides a new tool for quantitative proteomics. We demonstrate linear and reproducible quantification of proteins using PrISM, and the potential for this method to detect low abundance proteins and proteoforms in complex biological samples in the future.

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Abstract 2284

Elucidating FIBCD1 Binding Partners

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Min Xue

Fibrinogen C-domain containing 1 (FIBCD1) is a homotetrameric plasma membrane transport protein that contains a promiscuous receptor. The receptor has been reported to recognize a diverse set of substrates ranging from those containing acetylated and sulphated groups to carbohydrates. Once the molecule binds, the protein will facilitate endocytosis, however, the exact mechanism behind this is currently not well understood. FIBCD1 is also speculated to participate in pathogen recognition and as a result a stronger understanding of the protein should aid in the deciphering of how the immune system responds to the presence of various pathogens. Therefore, we are interested in obtaining a more comprehensive understanding of FIBCD1's structure-activity to elucidate additional functions. Several genes were noted to be upregulated with the expression of FIBCD1 and may also participate in the endocytic mechanism. CRISPR will be utilized to determine these observed relations. We also have focused on the C-terminal fibrinogen-like recognition domain (FReD), which encompasses amino acids 236–461. In addition, to facilitate these investigations we established a unique purification scheme for the truncated construct. We also intend to investigate the protein's potential interactions with bacterial glycans also to further unravel the protein's ability to recognize pathogens.

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ASBMB

Abstract 2317

Identifying novel cysteine metabolic fates in NRF2-activated cholangiocarcinoma cells

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The discovery of metabolites and metabolic pathways is amongst the most fundamental advancements in biological research. While the components of the major metabolic pathways are well described, there is no reason to assume that our current knowledge of human metabolism includes all existing metabolites in all tissue types and conditions. Recent discoveries of previously uncharacterized, context specific cancer-modifying metabolites, including 2-hydroxyglutarate in IDH mutant cancers, the glutathione-fumarate conjugate GSF in FH mutant kidney cancers, and S-geranylgeranyl-L-glutathione in germinal-center B cell-like diffuse large B cell lymphomas suggests that additional disease relevant, but currently unknown, metabolites likely exist. Identifying remaining metabolites is a critical goal towards improving our understanding of fundamental biology and may lead to novel therapeutics that can target metabolism in disease states. Towards this goal, we developed an untargeted isotope tracing protocol where cancer cells are fed nutrients containing isotopically labeled atoms, intracellular metabolites are extracted and measured by liquid chromatography mass spectrometry, and metabolic fates are identified using a data analysis pipeline that identifies downstream metabolites that incorporate the heavy atoms derived from the parent nutrient. Here, we deploy this technology to investigate the metabolic fates of cystine, the primary precursor of intracellular cysteine and a critical resource for cancer cell function. To identify cystine fates associated with cancer phenotypes, we conducted untargeted cystine tracing on a group of bile duct cancer cell lines, focusing on which cystine fates were enriched in cells with activation of the oncogenic transcription factor NRF2, which promotes cystine uptake and metabolism. Our approach identifies over 100 potential cystine fates in these cells, the majority of which are enriched in NRF2-activated cells. We then characterize several of the unknown cysteine fates that are most enriched in NRF2-activated cells and find that many derive from previously undescribed covalent conjugates between glycolytic intermediates and cysteine. We find that these molecules can also be generated in cell free systems, confirming their source and suggesting they may derive from non-enzymatic reactions in cells with excess cysteine. Further, we find that titrating the availability of cystine can modify the abundance of cysteine fates and alter glycolytic metabolism. Collectively, these findings identify a novel group of cysteine-derived metabolites enriched in specific disease states in bile duct cancer cell lines, supporting the hypothesis that currently unknown metabolites exist and may serve context specific roles in human disease.

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Abstract 2332

Long-term effects of simulated microgravity on protein SUMOylaiton and non-covalent SUMO interactions

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Rita Miller

Microgravity of space travel produces a profound stress on cells of the body, resulting in many health risks for astronauts. Dramatic changes in cell morphology and the cytoskeleton contribute to a host of physiological dysfunctions. However, the molecular mechanisms by which cells sense and respond to this stress are still poorly understood. SUMO is a small ubiquitin-like modifier known to respond to cell stress. SUMO can interact with proteins either through its covalent attachment to a target lysine or through its non-covalent interactions with a binding partner. SUMO is critical in many cellular processes, including for DNA damage repair, proper cytoskeleton regulation, cellular division, and protein turnover. Because these processes are also disrupted in microgravity, we hypothesized that SUMO regulates the cell's stress response to microgravity. To test this, we investigated the differences in protein steady-state levels and protein modifications in yeast grown in normal gravity and simulated microgravity. Specifically, *S. cerevisiae* was grown for six generations in a rotating wall vessel to generate a simulated microgravity condition, alongside a normal gravity control. SILAC labelling was used to distinguish the two conditions. Proteins were isolated and analyzed by mass spectrometry from 6 bio reps. Using these extracts, we asked three questions. First, we asked to what extent are proteins differentially conjugated by SUMO in gravity versus simulated microgravity? To identify these proteins, we employed a modified 6xHIS-tagged form of SUMO that is resistant to the protease Lys-C to isolate SUMOylated proteins. 347 covalently SUMOylated proteins were identified in this screen. The abundance ratios of SUMOylated proteins between gravity and simulated microgravity was calculated from the SILAC labeling. 18 SUMOylated proteins had a >50% increased/decreased protein abundance in simulated microgravity. Second, we asked to what extent are proteins bound differentially in a non-covalently manner to SUMO in gravity versus microgravity? To answer this question, we used three forms of GST-SUMO columns in which SUMO could only interact non-covalently with its binding partners. These column included a GST-SUMO-GA column, a SUMO-GST column, and a 5xSUMO-GST column. These columns were used to enrich the non-covalent SUMO interactome. Over 1,500 proteins were identified as interacting with at least one of the non-covalent SUMO columns. Of the 1,500 proteins, 19 are significantly increased/decreased >50%. Third, we asked what proteins in the cell's proteome are differentially abundant in gravity versus microgravity? To answer this question, SILAC labeled whole cell extracts were analyzed for protein differences by mass spectrometry. Of the 3,773 proteins identified in this

screen, 34 proteins decreased >50% and 8 increased >50% in simulated microgravity. These differentially abundant proteins are involved in DNA damage repair, cellular division regulation, histone acetyltransferase, ubiquitination, and SUMO mediated chromosome maintenance. Together, this data suggests that SUMO is a key factor in cellular adaptation to microgravity stress and provides insights at the cellular level about how cells manage the stress of microgravity.

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Abstract 2353

Advancing glycomics and glycoproteomics through innovation in chemical tagging approaches

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Gycosylation is one of the most important and most complex protein post-translational modifications. Alterations in glycomic profiles have been linked to various diseases, including cancer, neurodegenerative disorders, and cardiovascular problems. Thus, new methods are needed for quantitative analysis of glycans to facilitate elucidation of the diverse biological roles of glycans and their roles in human diseases. Advances in mass spectrometry (MS)-based glycoproteomics and glycomics are increasingly enabling qualitative and quantitative approaches for site-specific structural analysis of protein glycosylation. However, quantitative analysis of native glycans remains extremely challenging due to high complexity and diversity of glycan structures, difficulty of synthesizing glycan standards, the relatively low response in MS detection, and the wide dynamic range of glycans in clinically relevant samples. In this presentation, I will describe our recent efforts in developing chemical tags and quantification strategies for both N-glycan and intact glycopeptide analysis in biological samples. We designed and synthesized a set of 4-plex isobaric multiplex reagents for carbonyl containing compounds (SUGAR) tags for multiplexed MS2-level glycan characterization and relative quantitation. To further improve the throughput, we utilized a mass-defect concept where subtle mass differences can be introduced to different isotopologues and expanded the multiplexing capacity to 12 channels, a three-fold throughput enhancement for SUGAR tag-based quantitative glycomics. In parallel, we also develop chemical tags such as N, N-dimethylated leucine (DiLeu) for glycopeptide quantitation. Specifically, we improved the original DiLeu design and developed isobaric N, N-dimethyl leucine derivatized ethylenediamine (DiLeuEN) tags to increase the charge states of glycopeptides to achieve enhanced fragmentation efficiency and in-depth characterization of intact glycopeptides, especially sialoglycopeptides. Collectively, these cost-effective and novel mass defect-based labeling reagents enable robust, sensitive and accurate glycomic and glycoproteomic analysis with enhanced quantitative performance and structural elucidation capabilities. The performance and utility of these tags will be evaluated and demonstrated by several complex biological samples.

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ASBMB

Abstract 2359

Mass spectrometry-based metabolomics for investigation of chemical exposure in *Drosophila melanogaster*

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Kenia Contreras, Dorian Goolsby, Christina Rabban, Mustafa Azam, Seo-Yun Lee, Jacob Kagey, Charles Evans, Kendra Evans

Exposure to pesticides and other toxic compounds has been demonstrated to cause a variety of harmful health impacts and can ultimately result in disease and/or disease-like symptoms. To probe the role of altered metabolism in such disease states, we have developed methods for untargeted liquid chromatography-mass spectrometry-based metabolomics investigations of atrazine and ethyl methanesulfonate exposure in *Drosophila melanogaster*. The use of Drosophila as a model organism in the study of pesticide exposure has been demonstrated to be relevant to human health because similar alterations in metabolic pathways have been observed in both pesticide-exposed Drosophila and in postmortem samples of patients suffering from environmental pesticide exposure. Preliminary results from studies performed on control flies and flies fed a diet including the triazine-based herbicide atrazine for 12–24 h will be reported. Future work will include exploration of the physiological effects of pesticide exposure and the implications of any observed altered metabolism. In an additional investigation of ethyl methanesulfonate exposure, we aim to study whether exposure-induced changes in metabolism are affiliated with previously observed changes in the Drosophila genome and phenome post-exposure. Preliminary results performed on control flies and flies exposed to ethyl methanesulfonate for 16 h will be reported. Further analyses will be performed to explore potential impact of metabolic changes on alterations in the Drosophila genome and phenome.

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Abstract 2431

Hermansky-Pudlak Syndrome Type 2 (HPS-2) Patient's Fibroblasts Show an Increase in Fatty Acid Concentrations

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Ariana Reyes-Rosado, Nataliya Chorna, Carmen Cadilla-Vazquez

Hermansky-Pudlak Syndrome (HPS) is a rare autosomal recessive disorder in which the biogenesis of lysosome related organelles is affected. Up to date there are 11 subtypes of HPS (HPS-1 through HPS-11) each one arising from mutations in different genes that code for proteins in the AP-3 adaptor and Biogenesis of Lysosome Related Organelles Complexes (BLOCs). Clinical manifestations found in these patients include: oculocutaneous albinism, nystagmus, bleeding diathesis, granulomatous colitis, and pulmonary fibrosis. There is no cure for the pulmonary fibrosis found in HPS patients which may result in the need for lung transplantation. HPS subtypes that can develop pulmonary fibrosis are HPS-1 and HPS-4 (with mutations in BLOC3 components), and HPS-2 (with mutations in AP3B1). The molecular mechanisms that influence the development of pulmonary fibrosis are yet to be defined. Bronco-alveolar lavages (BAL) from patients have shown that an inflammatory response precedes the fibrosis. Therefore, we hypothesize that HPS patients with mutations in BLOC3 components and AP3B1 will have an increase in inflammatory lipids in comparison to control. To test our hypothesis we used primary fibroblasts from a patient with HPS-1 (GM14609), a patient with HPS-2 (GM17890), and a normal control donor (GM00037). We extracted the metabolites with 85% methanol solution and performed a two-step derivatization: methoxyamination and trimethylsilylation followed by fractionation by GC-MS. We used Metaboanalyst 5.0 for data analysis and normalized by log transformation and Range scaling. To determine differences between groups we used Partial Least-Squares Discriminant Analysis (PLS-DA) model after its validation using a permutation test (p value < 0.001). Levels of two fatty acids were significantly different between our sample groups: palmitic acid, and oleic acid. HPS-2 fibroblasts had the highest concentration of both fatty acids in comparison with control and HPS-1 fibroblasts. To test for biologically meaningful metabolites enriched in our data, we performed an enrichment analysis applying a p value of < 0.05 and selecting pathways with 2 or more entries. Between HPS-2 fibroblasts and HPS-1 fibroblasts the most enriched pathways were those involved in fatty acids biosynthesis. Control and HPS-2 fibroblasts analysis showed that groups of metabolites were significantly enriched in amino acids and fatty acids biosynthesis and degradation. These preliminary results suggest that HPS-2 patients could have some sort of dyslipidemia. A deep analysis on complex lipids by LC-MS should be performed to identify those lipids that could be influencing the development of pulmonary fibrosis in these patients.

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Abstract 2442

Quantitation of atrazine and its metabolites for toxicological studies in the keystone species *Faxonius virilis*

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Kenia Contreras, Dorian Goolsby, Reema Hamdan, Caela Fedraw, Andrew Bosah, Mariana Muskovac, Kendra Evans, Rachelle Belanger

Atrazine is a pesticide of the triazine class and is one of the most used pesticides in North America. The effects of atrazine exposure on crayfish are of interest because crayfish are a keystone species. Atrazine has many negative health effects such as disrupting regular hormone function, birth defects, and DNA damage in the hepatopancreas. Furthermore, atrazine can cause metabolic changes that diminish the ability for crayfish to localize an odor source, making it difficult to locate food and find a mate. Exposure to atrazine also affects the expression and activity of the detoxification enzymes cytochrome P450 and glutathione-S-transferase. To further explore the effects of atrazine exposure, crayfish (*Faxonius virilis*) were exposed to atrazine for four days. Liquid chromatography-mass spectrometry (LC-MS) was used to standardize the atrazine solutions to which crayfish were exposed. The morphology of the hepatopancreas of atrazine-exposed crayfish was examined; lobule lumens were dilated, and tubule tissue was degenerated and contained an increase in vacuoles. We developed and validated an extraction and LC-MS analysis method for quantitation of atrazine and its metabolites in hepatopancreas tissue. With this method, we aim to investigate accumulation and metabolism of atrazine in the hepatopancreas. Using these data, we will assess correlation between levels of atrazine and its metabolites with observed behavioral and physiological phenotype.

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Abstract 2560

Narrow Bottlenecks Constrain Influenza A Virus Genetic Diversity During Direct Contact Transmission

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Luis Haddock III Soto, Katherine Amato, Katarina Braun, Stacey Schultz-Cherry, Gabriele Neumann, Yoshihiro Kawaoka, Andrew Mehle, Thomas Friedrich

Influenza A viruses (IAV) within a host exist as dynamic and diverse populations in which related variants arise by low-fidelity replication. Transmission of IAV between hosts is subject to physical and biological barriers, imposing genetic bottlenecks that can sharply reduce IAV genetic diversity. Narrow bottlenecks during transmission reduce transmitted viral population size and diversity, so variants with potential replicative advantages may be lost. In contrast, loose bottlenecks allow for a bigger and more diverse viral population to be transmitted. Population bottlenecks, therefore, play an essential role in determining the evolutionary pathways taken by IAV on larger scales. Here we sought to characterize the mechanisms that govern IAV population bottlenecks within and between hosts. We created diverse IAV libraries bearing short stretches of noncoding nucleotide sequences, called molecular barcodes, on the hemagglutinin (HA) gene segment. Molecular barcodes were quantified via targeted deep sequencing, which allowed for high-resolution tracking and quantification of over 50,000 unique viral lineages. We performed site-specific inoculation of barcoded IAV in the upper respiratory tract of ferrets and then tracked viral diversity as the infection spread throughout the respiratory tract and by direct contact transmission to naïve hosts. We show that viral evolution within a host during IAV infections is influenced by population bottlenecks that result in anatomical compartmentalization, leading to genetically distinct populations throughout the respiratory tract. Barcode quantification and bottleneck size estimations reveal rich and diverse populations replicating in the upper respiratory tract that are stochastically sampled as viruses transit to the lung, resulting in dramatic expansions of distinct lineages in different lung lobes. We then use our barcoded viruses to study evolution during transmission between hosts. IAV transmission via direct contact markedly reduces viral diversity between donor and recipient animals. Only a small fraction of viral lineages are detected in the recipient immediately following exposure. Diversity within this population was further reduced, with only 1–3 lineages dominating viral populations in the recipient at later times post-exposure. These data suggest that infection in a new host is sustained by a small subset of viruses from the larger pool that is initially transferred from the donor. Our findings show that IAV diversity is shaped by stringent bottlenecks before and during transmission in both the donor and recipient hosts. Anatomical compartmentalization within a host and narrow transmission bottlenecks between hosts significantly lower the rates of adaptation during acute infection, resulting in stochastic viral evolution shaped predominantly by genetic drift and founder effects. We propose that these stringent bottlenecks act to slow the pace of influenza virus evolution.

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Abstract 2595

Multi-omic analyses identified sex-dependent effects of SARM1 deficiency from SARM1-KO mice

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Decline in cellular nicotinamide adenine dinucleotide (NAD+) levels causes diseases including heart disease by impairing energy metabolism, cell signaling, and mitochondrial function. Activating NAD+ synthesis to raise cellular NAD+ levels is therefore therapeutic. Alternatively, inhibition of NAD+ consumption may also maintain cellular NAD+ levels as a new therapeutic target. Unlike Sirtuins, Sterile-α and TIR motif containing 1 protein (SARM1) is an intracellular NAD+ hydrolase that does not directly regulate post-translational modification (PTMs). SARM1 therefore may be an attractive target to inhibit for maintaining cellular NAD+ levels without interfering PTMs. Using SARM1 knockout mice (KO) as a genetic model, it was reported that SARM1 mediates NAD+ decline in injury-induced axonal degeneration and cell death. However, the impacts of SARM1 deficiency on heart function and metabolism have never been investigated. Here we examined the impacts of SARM1 deficiency on cardiac function and metabolism in KO mice. Cardiac function was determined by echocardiography. Hearts were collected for transcriptomic analysis and NAD+ level determination. Plasma samples were collected for metabolomic analyses. Echocardiographic analysis indicated that neither male nor female KO mice exhibited significant changes in diastolic/systolic function and cardiac structure, compared to wild type, age-matched counterparts. We found that cardiac NAD+ pools increased in female KO hearts but unchanged in male KO hearts. Cardiac transcriptomic analysis showed more transcript expression changes (FDR < 0.05) in male KO hearts (293) than in female KO hearts (54), compared to wild type counterparts. Only 20 transcript expression changes were conserved in both sexes. These results suggested sexual dimorphic effects of SARM1 deficiency on cardiac NAD+ pool and transcriptome. Plasma metabolomic analyses measured 243 aqueous and lipid metabolites. Male KO plasma had 71 metabolites showed differences in levels, while female KO plasma only had 29. Principal component analysis further showed a greater degree of separation by genotype of KO male plasma metabolome than KO female plasma metabolome, suggesting another sexual dimorphism of SARM1 deficiency. How the sex differences observed in the hearts and plasma from KO mice impact cardiac NAD+ levels and the progression of cardiometabolic diseases remain to be determined. Our results identified sex-dependent changes of SARM1 deficiency from SARM1-KO mice and warrant further investigations. Detailed analyses of these changes will identify how SARM1-dependent sexual dimorphism may impact disease pathogeneses.

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ASBMB

Abstract 2662

Identification of next-generation biomarkers of Vivax Malaria and understanding disease severity using proteomics

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Shalini Aggarwal

Plasmodium vivax malaria is one of the most lethal infectious diseases, with 7 million infections annually. One of the roadblocks to global vivax malaria elimination is the lack of highly sensitive, specific, and accurate diagnostic tools to differentiate *P. vivax* from pan malarial parasites due to low parasitemia and lack of continuous culture. This study is a step forward to address this issue by comprehensive proteome analysis of the parasite and host proteome for diagnosis and prognosis, respectively. Metadata proteome analysis of clinical isolates of *P. vivax* using experimental data and literature published till 2019 gave us five recurring parasite proteins, Hypothetical protein (PVX_083555, priority 1), Plasmodium exported protein, unknown function (PVX_003545, priority 2), PvSTP1 (putative, PVX_094303, priority 2), tryptophan-rich antigen (Pv-fam-a, PVX_090265, priority 2), and Pv-fam-d protein (PVX_101520, priority 3). Furthermore, comprehensive peptide analysis of 28 parasite pellets and 21 plasma samples gave Phist protein (priority 3) as most abundant parasite protein present in parasite pellets as well as plasma samples. Additionally, comprehensive host proteome analysis using machine learning based elastic net regularized linear regression model and artificial neural network inference helped in identification of one or more, significantly altered host proteins (Serum Amyloid A2, SAA2; Leucine rich alpha-2 glycoprotein, LRG; and Hemopexin, HXP) capable of prognosing the disease. Both the parasite and host proteins were combined to develop an immunoassay using biolayer interferometry, capable of diagnosis and prognosis of the patient plasma samples with enhanced efficiency. This assay can be further developed into a dip-chip prototype for clinical translation of the findings, for efficient treatment and elimination of vivax malaria.

Department of Biotechnology, Government of India.

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Abstract 2697

Closed genome sequencing and pan-genome analysis of vaginal *Lactobacillus crispatus* isolates

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Chris Whidbey

The presence of *Lactobacillus crispatus* in the vaginal microbiome is associated with positive health outcomes, making it a potential probiotic candidate. However, the molecular mechanisms that contribute to these positive health outcomes remain elusive. Here, we describe whole-genome sequencing of vaginal isolates of varying species from partners at Baylor College of Medicine and NIH BEI using the Nanopore MinION platform. Closed genomes were then assembled and annotated. Pangenome analysis was performed on *L. crispatus* genomes. To better understand the mutualistic interaction between *Lactobacillus crispatus* and humans, a series of phenotypic assays were also performed. VSI04 indicated greatest sensitivity to low pH in a variable pH tolerance assay. Aggregation was observed qualitatively– strains VSI08, VSI24, and VC01 demonstrated low aggregation compared to VSI04, VSI17, PSS, 125-2-CHN, MV1AUS, or JVV01. By comparing aggregation in MRS against aggregation in culture supernatant, it was concluded that aggregation properties are likely to be due to cell-surface composition instead of secreted factors. As genomes become more widely available and annotated aggregation, pH tolerance, and adhesion variability can be correlated with gene presence.

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Abstract 2711

New Advances in Cross-linking Mass Spectrometry for Mapping Protein-Protein Interactions

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Protein-protein interactions (PPIs) are essential for the assembly of protein complexes, which are the active molecular modules for controlling various biological processes to maintain cell viability and homeostasis. Detailed analysis of PPIs at the systems-level will not only advance our understanding of cellular structures and functions as well as their associations with human pathologies, but also facilitate the exploration of novel interaction-based therapeutics. However, obtaining an authentic portrait of endogenous PPI networks has been a challenging task. In recent years, cross-linking mass spectrometry (XL-MS) has proven effective in global profiling of protein interaction networks. In comparison to other PPI methods, XL-MS is unique due to its capability of capturing native PPIs from cellular environments, and uncovering their identities and connectivity simultaneously without cell engineering. Despite its great potential, XL-MS analysis remains difficult in effective detection and identification of cross-linked peptides from complex samples. To advance XL-MS studies, we have developed a series of sulfoxide-containing MS-cleavable cross-linkers to enable simplified and accurate identification of cross-linked peptides. Our MS-cleavable reagents have been successfully applied to define global PPIs and elucidate architectures of protein complexes *in vitro* and *in vivo*. Here, we will present new developments of MS-cleavable XL-MS technologies and their applications to enable biological discoveries. The analytical platform described here can be directly adopted to study PPIs in any organisms and sample origins.

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Abstract 2716

The Prevalence of Rs5219 on the KCNJ11 Gene and Its Correlation to Type 2 Diabetes in the Hispanic/Latino Population in California

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In the United States, over 37 million individuals have diabetes, 90–95% of which have type 2 diabetes. The prevalence of type 2 diabetes (T2D) in America may be high, but the rate is exponentially higher for those belonging to U.S. minority groups. This elevated rate is dramatically apparent in the Hispanic/Latino population, making the disease the fifth leading cause of death for Hispanics/Latinos in the U.S. Single nucleotide polymorphisms (SNPs) are biomarkers caused by a variation at a single position in a DNA sequence. The rs5219 SNP, located on the KCNJ11 gene, correlates with T2D; the allele variation within rs5219 involves the A and G alleles. This project aims to detect the prevalence of rs5219 in 25 control and 25 diabetic Hispanic/Latino patients to determine the link between the rs5219 SNP and T2D in Orange County, California's Hispanic/Latino population. DNA was extracted from buccal swab samples and subjected to a polymerase chain reaction (PCR), followed by gel electrophoresis to confirm proper amplification. Enzymatic digestion was completed using the BanII enzyme to detect alleles based on restriction fragment length patterns. The collected data revealed the association of allele G with an increased risk of T2D. The knowledge gained from this study could provide awareness for Hispanic/Latino individuals with a heightened risk of T2D and aid in improving preventative action.

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ASBMB

Abstract 2725

Cardiac aging and heart failure with preserved ejection fraction induce distinct proteomic and phosphoproteomic remodeling in murine hearts

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Heart failure with preserved ejection fraction (HFpEF) is an emerging health problem with high morbidity and mortality and accounts for 50% of all heart failure cases. Currently, there is a lack of effective, evidence-based treatments for HFpEF and a better understanding of its molecular mechanisms is critical for development of new HFpEF therapies. The prevalence of HFpEF increases sharply with age but despite this strong association with advanced age, the impacts of aging on HFpEF pathogenesis have not been established. The objective of the study is to determine the impacts of aging on cardiac proteomic and phosphoproteomic remodeling during HFpEF. We employed a 5-week HFpEF-inducing regimen with a combination of metabolic (by high fat diet) and hypertensive (by L-NAME) stress, to induce HFpEF development in young and old mice. LC-MS/MS was performed on cardiac tissue samples before and after phosphopeptide enrichment to compare cardiac proteome and phosphoproteome of 1) young control, 2) young HFpEF, 3) old control and 4) old HFpEF mice. Our proteomic analysis showed that while aging induced a drastic proteomic remodeling in the heart, HFpEF development induced modest cardiac proteomic changes at both young and old ages. This suggests that HFpEF and aging mediate cardiac dysfunction through different mechanisms at proteomic levels. Our phosphoproteomic analysis found that while both aging and HFpEF development were accompanied by a significant remodeling of cardiac phosphoproteome, the phosphoproteomic changes induced by aging were different from the changes induced by HFpEF development. Interestingly, HFpEF development induced differential phosphoproteomic changes at the two age groups. For example, young HFpEF mice exhibited reduced levels of phosphorylation of T172 of AMP-activated protein kinase (AMPK2) compared to young control mice but old HFpEF mice exhibited increased T172 phosphorylation of AMPK2 compared to old control mice. The differential HFpEF-induced phosphoproteomic remodeling in the two age groups suggests that certain molecular mechanisms of HFpEF could be age-dependent. Together, our results support the importance to investigate the specific impacts of aging on HFpEF development and to identify molecular mechanisms of HFpEF using old HFpEF models.

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Abstract 2748

Protein folding stabilities are a major determinant of oxidation rates for buried methionine residues

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The oxidation of protein-bound methionines to form methionine sulfoxides has a broad range of biological ramifications, making it important to delineate factors that influence methionine oxidation rates within a given protein. This is especially important for biopharmaceuticals, where oxidation can lead to deactivation and degradation. Previously, neighboring residue effects and solvent accessibility (SA) have been shown to impact the susceptibility of methionine residues to oxidation. In this study, we provide proteome-wide evidence that oxidation rates of buried methionine residues are also strongly influenced by the thermodynamic folding stability of proteins. We surveyed the *E. coli* proteome using several proteomic methodologies and globally measured oxidation rates of methionine residues in the presence and absence of tertiary structure, as well as the folding stabilities of methionine containing domains. These data indicated that buried methionines have a wide range of protection factors against oxidation that correlate strongly with folding stabilities. Consistent with this, we show that in comparison to *E. coli*, the proteome of the thermophile *T. thermophilus* is significantly more stable and thus more resistant to methionine oxidation. To demonstrate the utility of this correlation, we used native methionine oxidation rates to survey the folding stabilities of *E. coli* and *T. thermophilus* proteomes at various temperatures and propose a model that relates the temperature dependence of the folding stabilities of these two species to their optimal growth temperatures. Overall, these results indicate that oxidation rates of buried methionines from the native state of proteins can be used as a metric of folding stability.

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Topic Category Glycans and Glycobiology

Abstract 1215

Characterization of the Transcriptome of the Drosophila Larval Salivary Glands at a Single-Cell Resolution

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Defects in secretion cause a variety of diseases, such as inflammatory bowel disorders, cystic fibrosis, and syndrome of inappropriate secretion of antidiuretic hormone. *Drosophila melanogaster* salivary glands provide a unique, *in vivo* system to study secretion. Third-instar larval salivary gland secretion happens in a distinct spatiotemporal pattern, where exocrine cells package and secrete large, highly glycosylated proteins such as mucins, under the regulation of steroid hormone 20-hydroxyecdysone (20E). In this study, using single-cell RNA sequencing, we characterized the gene expression of individual cell types in the Drosophila larval salivary glands at different development stages. Analysis of differentially expressed genes highlighted a variety of pathways and manners in which secretion may be disrupted, including dysregulation of the spatiotemporal secretion pattern and defects in secretory granule maturation. Further exploration of differentially expressed genes highlighted possible antimicrobial peptide transcription during salivary gland development. Finally, we are seeking to identify unknown cell populations found from the single cell analysis using fluorescent in-situ hybridization (FISH). In the future, deeper analysis of scRNA-seq data and further investigation of gene functions in different cell populations will allow better understanding the secretory granule biogenesis and regulated secretion.

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Abstract 1295

A novel technique for identifying polysialylated proteins in complex mixtures

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Polysialic acid (polySia) is a large homopolymer of alpha-2,8-linked Neu5Ac residues which has profound consequences for the proteins it is attached to. In healthy human adults, polySia is found in the nervous, reproductive, and immune systems where it contributes to cell migration and reduces the immune responses. PolySia is also abnormally expressed in chronic health conditions, including mental health disorders, auto-immune diseases, and cancers. Its expression is strongly correlated to poor prognoses. However, the mechanisms underlying polySia biology are poorly understood, in part because we do not know what proteins are polysialylated. Currently, there remains less than half a dozen identified polysialylated proteins and our analyses indicate that there are many more which have yet to be discovered. Being able to identify polysialylated proteins in complex mixtures, such as cells or serum, is a crucial first step in understanding the role of polySia in health and disease. We have developed novel methodology to identify which proteins in a complex mixture are polysialylated. In this strategy, we have biotinylated a polySia lectin in order to immobilize it on streptavidin agarose. The streptavidin agarose beads can be added to complex mixtures such as cell lysate or serum to isolate the proteins. These isolated proteins undergo protein identification using mass spectrometry. This new methodology substantially improves upon traditional methods like immunoprecipitation because it allows for stringent washing of the captured proteins with detergents to remove non-specifically bound proteins. We have demonstrated the utility of this method by correctly identifying neural cell adhesion molecule (NCAM) as a polysialylated protein in the non-Hodgkin's lymphoma cell line NK-92. In addition to improving our understanding of the role polySia plays in health and disease, these identified proteins have the potential to be used as biomarkers for diagnostic and prognostic testing. This proteomics method is versatile and will be useful for identifying polysialylated proteins from various sources such as immune cells, cancers, and other diseases.

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ASBMB

Abstract 1312

New tools to study polysialic acid reveal it is dysregulated in the autoimmune disease scleroderma

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Polysialic acid (polySia) is normally limited to the immune, nervous, and reproductive systems of healthy human adults, where it plays pivotal roles in cell migration and attenuation of immune responses. It is also dysregulated in chronic diseases like cancer, where increases in polySia are strongly associated with increased metastasis and poorer prognosis. However, the biochemical properties of polySia make it difficult to study and as a result, the mechanisms supporting polySia function in health and disease remain largely unknown. We developed several new methodologies which can be used to study polySia. First, an ELISA assay that allows for the detection and quantification of polySia, polysialylated proteins, and polysialylated extracellular vesicles. Second, affinity purification strategies which allow for the robust identification of polysialylated proteins using mass spectrometry. Third, a metabolic inhibitor of alpha-2,8-linked sialylation, called 8-keto-sialic acid. Using these techniques, we discovered a role for polySia in the pathogenesis of scleroderma, a rare but devastating autoimmune disease that predominantly affects women. These tools will substantially improve our ability to study polysialic acid, and may provide templates for new tools to study related glycans.

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Abstract 1368

Discovery and characterization of C5-methylpyrimidine DNA hypermodification pathways

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Structural and chemical diversity of DNA is introduced by enzyme-catalyzed chemical modification of the canonical bases at either the individual nucleotide or DNA polymer level. These modifications expand the chemical functionality of DNA, analogous to post-transcriptional modifications in RNA and post-translational modifications in proteins. DNA base modifications afford physiological roles including epigenetics and protection against endonuclease cleavage (bacteriophage systems). In addition to their biological roles, the unexplored DNA base modification systems in nature potentially hold a wealth of novel chemistries that could be utilized in new biotechnological and biomedical applications. The Saleh group at New England Biolabs is investigating the chemical and structural diversity of hypermodifications installed at the C5 position of 5-methylpyrimidines through rational exploitation of "genome neighborhood" information in metagenomic databases using in silico-guided strategies for functional discovery. Leveraging these in silico predictions, we employ high-throughput strategies for enzyme discovery and characterization to analyze these biosynthetic pathways. Through extensive bioinformatic and biochemical studies, we have identified a post-replicative pathway for DNA hypermodification in bacteriophage that utilizes an Fe(II)/2-oxoglutarate-dependent 5-methylpyrimidine dioxygenase (5mYOX) to install a reactive handle on the C5-position of methylpyrimidines. Further enzymatic modification of C5-hydroxymethylpyrimidines produces a diversity of DNA base modifications (Burke, E. J. et al. (2021) PNAS, 118(26): e2026742118). Combining phylogenetic classification with biochemical characterization of 5mYOX homologs, we gain insight into the 5mYOX superfamily that catalyze oxidation of 5-methylpyrimidine to 5-hydroxymethylpyrimidine. We also describe novel glycosyl-modifications on cytosine and elaborate on the chemistry of glycosyltransferase-associated biosynthetic clusters that install them. Overall, we present a powerful biochemical strategy for exploring modifications on DNA and for understanding the enzymes that install them.

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Abstract 1388

Structural basis for heparan sulfate co-polymerase action by the EXT1-2 complex

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Proteoglycans harboring heparan sulfate (HS) chains are ubiquitously expressed on cell surfaces and in extracellular matrices. These glycosaminoglycan chains interact with numerous proteins, growth factors, morphogens, and extracellular matrix proteins and play crucial roles in tissue homeostasis and signal transduction, where they drive processes such as cell survival, division, migration, differentiation, pathogen binding, and cancer development. Heparan sulfate (HS) proteoglycans contain extended (GlcAβ1,4GlcNAcα1,4-)n copolymers containing decorations of sulfation and epimerization that are linked to cell surface and extracellular matrix proteins. In mammals, HS repeat units are extended by an obligate heterocomplex of two exostosin family members, EXT1 and EXT2, where each protein monomer contains distinct GT64 (GT-B fold) and GT47 (GT-A fold) glycosyltransferase domains. Thus, each isoform could theoretically contribute both GlcA and GlcNAc transferase activities, although no studies have yet determined which sites in the EXT1-2 complex are indeed functional. We have expressed human EXT1-EXT2 (EXT1-2) as a functional heterocomplex and determined its structure in the presence of bound donor and acceptor substrates. Structural data and enzyme activity of catalytic site mutants demonstrate only two of the four glycosyltransferase domains are major contributors to co-polymer syntheses: the EXT1 GT-B fold β1,4GlcA transferase domain and the EXT2 GT-A fold α1,4GlcNAc transferase domain. Thus, each protein contributes one catalytic site. The two catalytic sites of the co-polymerase complex are over 90 Å apart, indicating that HS is synthesized by a dissociative process in which the growing glycan switches back and forth between the two catalytic sites.

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Abstract 1509

Targeting Spike Glycans to Inhibit SARS-CoV-2 Viral Entry

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SARS-CoV-2 Spike harbors glycans which function as ligands for lectins. Therefore, it should be possible to exploit lectins to target SARS-CoV-2 and inhibit cellular entry by binding glycans on the Spike protein. *Burkholderia oklahomensis* agglutinin (BOA) is an antiviral lectin that interacts with viral glycoproteins via N-linked high mannose glycans. Here, we show that BOA binds to the Spike protein and is a potent inhibitor of SARS-CoV-2 viral entry at nanomolar concentrations. Using a variety of biophysical tools such as SEC chromatography, dynamics light scattering, fluorescence binding assays, and electron microscopy, we demonstrate that the interaction is avidity driven and that BOA crosslinks the Spike protein into soluble aggregates. Furthermore, using virus neutralization assays, we demonstrate that BOA effectively inhibits all tested variants of concern as well as SARS-CoV-1, establishing that glycan-targeting molecules have the potential to be pan-coronavirus inhibitors.

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Abstract 1524

Modular Economical One-Pot Multienzyme Synthesis of Complex Glycans

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Glycans are ubiquitous and play essential roles in diverse biological functions. Glycans have broad applications, including supplementation of human milk oligosaccharides to infant formula, design of glycan-based vaccines against pathogens and cancers, and functions of homogeneous glycoproteins, among many others. However, present synthesis methods fail in affordable scalability which hinders the development of glycan therapeutic applications. Even the wide-use FDA/EMA-approved pentasaccharide anticoagulant, fondaparinux, requires about 50 steps to be chemically synthesized, giving around 0.1% overall yield. Current one-pot multienzyme (OPME) systems have streamlined numerous steps in glycan synthesis; still, few have focused on their optimization and scalability, especially when integrating energy regeneration. In this work, we succeed in using a stable, affordable phosphate energy source to cycle different nucleotide sugars in situ for synthesizing complex glycans in one pot. Given the acidic and cation-sequestering nature of phosphate donors, large numbers of components in the reactions and their cross-interactions need to be assessed and optimized. We first enumerated all the possible factors and ranked their importance based on our understanding of the respective enzymology. Hard-to-change factors like different enzyme homologs have the lowest priority. We also established an efficient workflow to rapidly monitor the reactions at multiple time points by MALDI-TOF mass spectrometry; an in-house Python script was written to analyze and visualize countless mass spectra automatedly. Additionally, methods using design of experiments were adopted to systematically optimize the combined reaction mixture. Here, we demonstrate our workflow with the extension of lactose with β1,3-GlcNAc starting from inexpensive precursors and catalytic amounts of nucleotides. We have expanded and modularized our economical OPME system (ecOPME) into different sugars and linkages. Reduced input of nucleotides, especially expensive CTP and GTP, use of high sugar precursor concentrations, nearly 100% completion of the reactions, and minimal downstream repetitive purifications pave the path to multi-gram scale synthesis of complex glycans in an ordinary lab setup. Facile accessibility to complex glycans can facilitate research on their functions and applications that improve human health.

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Abstract 1535

Application of human oral lectin ZG16B as a microbial glycan analysis probe (mGAP) to decode host-microbe interactions

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The oral cavity, one of the primary interfaces for host-microbe interactions, features complex microbiota. The interaction between the oral microbiome with the host often involves the recognition of microbial cell wall carbohydrates by the carbohydrate-binding proteins, lectins being one of them. Zymogen granule protein 16 homolog B (ZG16B) is one such less explored human soluble lectin, expressed in high abundance in submandibular and sublingual glands in the oral cavity. To elucidate the function of ZG16B, we generated a microbial glycan analysis probe deploying recombinant lectin conjugated to fluorophore or biotin and applied it to interrogate the interactions of ZG16B with the oral microbiome using a replica-plating assay. Cy5 conjugated ZG16B was shown to bind to the cell wall peptidoglycan of oral commensal *Streptococcus vestibularis* isolated from dental plaques. ZG16B showed a bacteriostatic effect on *S. vestibularis* by aggregating the microbes, but not on other non-binding oral Streptococci, e.g. *Streptococcus oralis*. We further demonstrated that ZG16B binds to salivary mucin MUC7, but not to other predominant salivary mucins. ZG16B forms a ternary complex *in vitro* by recruiting MUC7 on *S. vestibularis*, enhancing clustering among the microbes. Our data suggest that ZG16B possibly regulates the overgrowth of oral commensals on the tooth surface by aggregation, followed by mucus-assisted clearance. The function of ZG16B reveals one of the possible mechanisms for maintaining bacterial homeostasis in the oral cavity through selective retention and regulation of bacteria via lectin-mediated host-microbe interaction.

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Abstract 1540

O-GlcNAc glycosylation orchestrates fate decision and niche function of bone marrow stromal progenitors

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Zengdi Zhang, Zan Huang, Hai-Bin Ruan

In mammals, interactions between the bone marrow (BM) stroma and hematopoietic progenitors contribute to bone-BM homeostasis. Perinatal bone growth and ossification provide a microenvironment for the transition to definitive hematopoiesis; however, mechanisms and interactions orchestrating the development of skeletal and hematopoietic systems remain largely unknown. Here, we establish intracellular O-linked β-N-acetylglucosamine (O-GlcNAc) modification as a posttranslational switch that dictates the differentiation fate and niche function of early BM stromal cells (BMSCs). By modifying and activating RUNX2, O-GlcNAcylation promotes osteogenic differentiation of BMSCs and stromal IL-7 expression to support lymphopoiesis. In contrast, C/EBPβ-dependent marrow adipogenesis and expression of myelopoietic stem cell factor (SCF) is inhibited by O-GlcNAcylation. Ablating O-GlcNAc transferase (OGT) in BMSCs leads to impaired bone formation, increased marrow adiposity, as well as defective B-cell lymphopoiesis and myeloid overproduction in mice. Thus, the balance of osteogenic and adipogenic differentiation of BMSCs is determined by reciprocal O-GlcNAc regulation of transcription factors, which simultaneously shapes the hematopoietic niche.

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Abstract 1549

Mucins form a nanoscale material barrier against immune cell attack

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Matthew Paszek

Cancer cells construct a glycocalyx with biochemical and physical attributes that protect against immune surveillance. Whether the structural properties of the glycocalyx also physically shield cancer cells from immune recognition has not been fully resolved. Here, we have developed an interference-based imaging tool called Scanning Angle Interference Microscopy (SAIM) to image the nanoscale physical dimensions and structural organization of the cellular glycocalyx. To improve the precision of SAIM for glycocalyx research, we utilize a pair of high-speed, galvanometer-controlled mirrors to generate a revolving circle, or "ring," of excitation light at defined sample incidence angles (Ring-SAIM). By combining genetic approaches and the imaging tools, we reveal how the surface density, glycosylation, and crosslinking of cancer-associated mucins contribute to the nanoscale material thickness of the glycocalyx, and further analyze the effect of the glycocalyx thickness on resistance to effector cell attack. We uncover a strong reciprocal relationship between the thickness of the glycocalyx and immune cell killing. Natural Killer (NK) cell-mediated cytotoxicity exhibits a nearly perfect inverse correlation with the glycocalyx thickness of target cells regardless of the specific glycan structures present, suggesting that the physical properties of glycocalyx may be key determinants of cancer immune evasion. Similar relationships were found for chimeric antigen receptor (CAR) NK cells against target cells with engineered glycocalyces. We further suggest strategies for overcoming the glycocalyx physical barrier through the cellular engineering of immune cells. Using leucine zipper-based conjugation approach, we generate engineered NK cells that tether glycocalyx-editing enzymes (GE) on the NK cell surface for improved penetration of the glycocalyx barrier and therefore induce higher NK cell-mediated killing.

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ASBMB

Abstract 1557

Engineering the *Neisseria meningitidis* Serogroup W Capsule Polymerase with Novel Substrate Specificity for Production of Novel Polysaccharides

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Trinity Bolton, James Wachira, Pumtiwitt McCarthy

The growing rate of environmental pollution by heavy metal is of great concern. This pollution has adverse effects on the health of humans, animals, and plants alike. Bacterial-derived polysaccharides such as those from *Neisseria meningitidis* are capable of adsorbing metals and can offer novel tools for environmental metal remediation. Our long-term goal is to use protein engineering approaches to change specificity of the *Neisseria meningitidis* serogroup W (NmW) capsule polymerase to produce novel N-acetylgalactosamine-sialic acid containing polymers and assess whether these sugars have increased metal-binding capacity. In our previously published work, potential amino acid positions that could alter the substrate specificity were identified. Here, we describe our progress of initial site-directed mutagenesis mutational studies to impart new specificity into the NmW capsule polymerase. Appropriate primers were designed for site directed mutagenesis of the NmW capsule polymerase with R234D (arginine to aspartic acid) and S306K (serine to lysine) mutations, respectively, using NEBaseChanger software. PCR amplification was performed and the resultant DNA, as visualized by gel electrophoresis, showed bands that correspond to the correct base pair (bp) sizes for both mutations. Further confirmation of the mutation by Sanger sequencing showed that only the R234D site-directed mutagenesis was successful. The R234D-containing plasmid was transformed into BL21(DE3) competent *E. coli* cells for expression. Colonies obtained from transformation were grown in LB-Ampicillin broth and samples were collected pre- and post- induction with IPTG. Post-induction hourly samples indicated a protein band with the desired molecular weight of the NmW capsule polymerase (120 kDa). Attempts at purifying the mutant protein from the bacterial cell lysate by nickel chromatography proved unsuccessful. Future work seeks to test the cell pellets and determine if the protein with the desired molecular weight is present in inclusion bodies.

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Abstract 1606

Structural Insight into an Anti-tumor Antibody Bound to a MUC1 Glycopeptide

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Angham Ahmed, Ricardo Cortez, Carina Amaya, Cory Brooks

While the cancer mortality rate in the United States continues to decline, cancer remains the second leading cause of death across the nation. Over the past number of years, monoclonal antibodies (mAbs) have emerged as alternative pharmaceuticals and have been utilized as regimens for the treatment of cancer. In noncancerous cells, the glycoprotein mucin 1 (MUC1) serves as a physical barrier against invading pathogens and acts as a lubricant for epithelial cells. However, in malignant cells MUC1 exhibits truncated and atypical glycosylation, leaving traditionally inaccessible epitopes exposed. Possessing high antigen affinity, tumor specificity, and an increased number of antibody binding sites, the MUC1 specific antibody, PankoMab (Gatipotuzumab), is a humanized mAb under investigation as an immunotherapy for the treatment of ovarian cancer. The antibody has increased selectivity for Tn-glycosylated MUC1. We aim to elucidate the structure of PankoMab in complex with a MUC1 glycopeptide in order to determine the mechanism behind the selectivity of the mAb for the glycosylated antigen. Recombinant PankoMab was produced as both a Fab fragment and IgG. The genes for the antibody light chain and heavy chain were cloned into the pcDNA 3.1 using a modular cloning approach mediated by Gibson assembly. Antibody was produced by transient transfection in expiCHO cells. Protein expression was confirmed by SDS PAGE and purified using Nickel or Protein A affinity chromatography. Interaction of the protein with 5 copies of the MUC1 tandem repeat fused to GST was confirmed by ELISA. The binding kinetics of the Fab fragment for a MUC1 peptide and MUC1 Tn glycopeptide were determined by SPR confirming the selectivity of the protein for the glycopeptide form of the antigen. Going forward, purified PankoMab Fab will be complexed with a MUC1 peptide and glycopeptide to determine the structural basis for the mechanism. Ultimately, a structural understanding of PankoMab Fab and its binding mechanisms to MUC1 may contribute towards the advancement of antibody-mediated immunotherapies.

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Abstract 1637

Adventures in the Biological Chemistry, Molecular Biology and Evolution of Sialic Acids

Ajit Varki, *University of California, San Diego*

Apparently immutable laws of mathematics, physics and chemistry were co-opted by biological evolution via natural selection, generating all known life forms from a last universal common ancestor. Some of the evolutionarily more ancient aspects of biological chemistry (e.g., glycolysis) and molecular biology (e.g., the genetic code and the central dogma enunciated by Francis Crick that genetic information flows from DNA, to RNA, to protein, or RNA to protein) are highly conserved due to long-term purifying selection that (along with fractal patterns) gives the illusion of "design." But, as Crick himself later opined, "there are no laws in biology, only gadgets." Given that the genetic code represents generation of order out of chaos, it is not surprising that the ongoing inter-species interactions of rapidly evolving pathogens and symbionts with hosts has resulted in the emergence of less conserved structures and functional phenomena. Of all the macromolecules in nature, the most diverse and rapidly evolving are glycans, which are also the least studied and understood—effectively "the dark matter of the biological universe." Among vertebrate glycans, some of the most diverse are sialic acids, which are prominently displayed at the outermost end of glycan chains in the deuterostome lineage of animals, and on pathogens and symbionts that interact with them. My group has spent many years studying the biological chemistry and molecular biology of sialic acids and sialoglycan-recognizing proteins in evolution, with a particular emphasis on human origins and disease. From the earliest days of my education in biology and medicine, the Journal of Biological Chemistry (JBC) had a major impact on my intellectual development as a physician-scientist. In this lecture I will cite a few such examples, with some focus on instances where multiple (often back-to-back) papers in the JBC helped focus attention on functionally significant variations of sialic acids, their glycosidic linkages, modifications such as O-acetyl and N-glycolyl groups, free amines and sulfate esters, and various combinations thereof. I will also briefly mention my limited but truly memorable interactions with Herb Tabor in the early days of online and open-access publishing, and a failed attempt to establish a uniform code of ethics for editors of biomedical journals.

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Abstract 1667

Profiling of O-fucose proteome using fucose analog

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O-fucosylation is an unusual glycan modification in which the fucose is linked directly to serine or threonine residues within specific consensus sequences on two cysteine-rich domains: Epidermal Growth Factor-like (EGF) repeats and Thrombospondin Type 1 Repeats (TSRs), mediated by Protein O-fucosyltransferases 1 (POFUT1) and 2 (POFUT2), respectively. O-fucose modulates the biological function of many proteins, including the Notch receptor, where proper O-fucosylation is required for Notch trafficking and ligand interaction. Disruption of cellular O-fucosylation is associated with many human congenital disorders and various types of cancer, highlighting the need to understand O-fucose proteome. Database searches with POFUT1 consensus sequence (C2xxxx(S/T)C3) in the context of an EGF repeat reveal that 87 proteins are predicted to be modified by POFUT1. Similarly, using POFUT2 consensus sequence (C1xx(S/T)C2) in the context of a TSR, 49 proteins are predicted to be modified by POFUT2. However, the fucosylation of most predicted proteins remains unconfirmed. Besides, recent data shows evidence that domains other than EGFs or TSRs can be O-fucosylated, raising the possibility that proteins outside the list can be modified. To address this need, we designed an unbiased glycoproteomic workflow for in-depth profiling of O-fucose proteome called "Direct detection of Fucose Analog-Tagged peptides" (DidFAT). We have demonstrated previously that 6-alkynyl fucose (6-AF) is efficiently incorporated into O-fucosylated proteins by POFUT1 and 2. Here we leveraged bioorthogonal click chemistry to couple 6-AF with a cleavable biotin linker (Diazo biotin azide). Chemical cleavage allows specific release of captured biotinylated peptides and leaves a signature residue on the peptides. We observed this residue displays unique mass spectrometric features by generating a high-intensity, O-fucose-specific diagnostic ion, which greatly improves glycoproteomic analysis. In a proof-of-principle experiment with expressed ADAMTS9 in HEK293T cells, we showed robust enrichment of peptides from all fucosylated TSRs in the cell secretome using DidFAT, with over 90% of the PSMs identified with the diagnostic ion. Besides ADAMTS9, a handful of endogenous proteins were identified with fucose on their predicted sites. Moreover, unexpected O-fucose sites were identified on Fribrillin-2 in a noncanonical consensus sequence, and Agrin, which may present an unassigned EGF domain. We are currently working on profiling O-fucose proteome in cell lysates, where more targets are expected to be identified. We believe DidFAT will provide a much deeper coverage of O-fucose proteome and discover novel targets to study.

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Abstract 1701

Biosynthetic Production of Non-native Glycans in *Escherichia coli* for Assembly and Study of *Campylobacter jejuni* Surface Oligosaccharide

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Jerry Troutman

Bacteria produce many types of biomolecules on the surface of their cell membranes that serve a wide range of functions such as sensing nutrient sources, adherence to a surface, and formation of biofilms. Many of these biomolecules are glycans that interact with host organisms. Interactions between bacterial glycans and host organisms can be beneficial (with anti-inflammatory effects) or be harmful (causing illness). When a bacterium uses enzymes embedded in its cell membrane to assemble a glycan, this is called biosynthesis. It is possible to copy the genetic material from a desired bacterium's chromosomal DNA and assemble it in an engineered bacterial plasmid, called a vector. When this vector is transformed, or inserted, into a viable strain of *Escherichia coli*, it can be trained to produce a desired glycan. Previous work in the Troutman lab has shown that entire biosynthesis pathways can be introduced through plasmids to *E. coli* to produce fully assembled bacterial glycans. Using *E. coli* as cell factories of biomolecules provides a means to bypass many steps that would be needed to chemically synthesize them. Four vectors were designed and assembled to start a library of *E. coli* that produce intermediate building blocks (uridine-diphosphate and undecaprenyl-diphosphate linked sugars) that can be combined to form fully functional glycans from the bacterial strain *Campylobacter jejuni*. Current work on this project is to confirm successful creation of each vector and to troubleshoot the production and harvesting of the resulting intermediate glycans. This fully assembled surface oligosaccharide from *C. jejuni*, along with its intermediate components, can be used to confirm enzyme activity as well as in potential development of vaccines that would instill immunity against host-cell interactions that cause disease.

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Abstract 1740

Evolution of fold-A glycosyltransferases and GTXplorer, a new tool for comparative glycomics

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Glycosylation is a common post-translational modification performed by Glycosyltransferases (GTs), a large and diverse superfamily which catalyze glycosidic linkages between sugars and other macromolecules. All GTs can be categorized by catalytic mechanism: whether they "invert" or "retain" the stereochemistry of the glycosidic bond. GTs can also be categorized into three distinct folds (A, B, or C), distinguished by their structure and subcellular location. We previously proposed a phylogeny of GT-A fold enzymes across the tree of life and uncovered a common hydrophobic core conserved amongst all GT-As. The hydrophobic core is typically associated with protein folding and stability, but little is known about how it shapes GT-A evolution and function. Using hidden Markov models and protein structural alignments, we uncover that the GT-A hydrophobic core evolved over three modules. The first module is an ancient Phosphate Binding Cassette (PBC) common to GT-As and unrelated nucleotide-binding proteins. This PBC is critical for coordinating the donor substrate of GTs. Next, the PBC is elaborated upon by the Rossmann N-lobe, preserved amongst Rossmann-fold enzymes. Third, GT-As diverged from other Rossmann fold enzymes through addition of two unique residues in the hydrophobic core that tether the PBC to the catalytic base (xED-Asp), linking donor and acceptor substrates in the active site. Conservation of this C-lobe tether is variable, correlating with the inverting and retaining mechanisms of GT-As. To evaluate the structural and functional impact of C-lobe tether variations, we hone in on one GT-A family, B3GNT2, and perform mutagenesis and molecular dynamics simulations. We find core mutations (T336I in B3GNT2) increase catalytic efficiency of the enzyme by shifting the conformational occupancy of the xED catalytic base. Specifically, we show two distinct conformations of the xED-asp, a core-facing "D-in" conformation and an acceptor-accessible "D-out" conformation, where increased access to the D-out conformation correlates with an increase in catalytic efficiency. Our studies support a model in which the core of GT-As evolved through progressive structural elaboration upon an ancient PBC, where plasticity of the hydrophobic core served as an advantageous framework that contributed to the remarkable diversity of catalytic and substrate-binding functions exhibited by extant glycosyltransferases. Finally, we present a new tool, GTXplorer, for biomedical scientists and protein engineers to navigate GT-A evolutionary information for hypothesis generation and investigation. An interactive GT-A phylogenetic tree visualizes

evolutionary relationships of GT-A families. Users may select relevant families on the tree and compare them at the residue level using annotated sequence logos specific to the selected clade, family, or subfamily. The GTXplorer webserver can be accessed at https://vulcan.cs.uga.edu/gtxplorer/ and represents the first evolutionary data analytics platform for comparative glycomics.

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Abstract 1800

The Role of TonB in Polysaccharide Utilization by *Bacteroides thetaiotaomicron*

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The human gut microbiota is required for the degradation of otherwise undigestible polysaccharides. These polysaccharides are a key energy source for the gut microbiota and fermentation products such as short chain fatty acids are beneficial to the human host. The Bacteroides are prominent contributors to polysaccharide degradation and TonB-dependent transporters (TBDT) are a key component responsible for uptake of polysaccharides. Transport through TBDT is energized by an inner membrane complex composed of TonB, ExbB, and ExbD which harnesses proton motive force and contacts the TBDT through TonB. *Bacteroides thetaiotaomicron* (B. theta) encodes 11 TonB homologues but it is not clear which homologues are important for polysaccharide uptake. To address this question, we have generated B. theta strains in which each of the 11 tonB genes are deleted. Using these single deletion strains, we have shown that TonB4 (BT2059) is important but not essential for proper growth on starch. Using membrane proteomics, we have shown an increase in abundance of TonB6 (BT2762) when TonB4 is absent, suggesting redundancy in function of these TonB proteins. Additionally, growth of the single deletion strains, on pectic galactan, chondroitin sulfate, arabinan, and levan suggest a similar redundancy in TonB function. Comparison of the B. theta TonB proteins to those encoded by other Bacteroides species suggests that TonB4 is widely conserved and may play a common role in polysaccharide uptake as recently shown in *B. fragilis*. However, conservation of TonB6 is much more limited suggesting that this redundancy of TonB function may be unique to B. theta and closely related species. This mechanistic understanding of how B. theta and related Bacteroides use polysaccharides to establish their niche in the microbiota will allow us to design non-invasive approaches for optimizing the gut community and improving patient outcomes caused by a lack or overgrowth of Bacteroides.

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Abstract 1821

Regulation of N-glycosylation by the PI3K/AKT signaling pathway

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The PI3K/AKT signaling pathway, which is frequently dysregulated in cancer, controls key cellular processes such as survival, proliferation, metabolism, and growth. Additionally, protein glycosylation is essential for proper protein folding and is also frequently deregulated in cancer. Cancer cells require increased protein folding capacity to sustain increased proliferation. The glycosyltransferase Asparagine-linked glycosylation 3 homolog (ALG3) catalyzes the addition of mannose units to a precursor during glycan biosynthesis. ALG3 is a rate-limiting enzyme during glycan biosynthesis. We found that in cancer cells, ALG3 is phosphorylated downstream of the PI3K/AKT pathway, and is directly phosphorylated by AKT at Ser11/Ser13. This represents a direct link between PI3K/AKT oncogenic signaling and protein glycosylation. Additionally, we found that ALG3 resides proximal to the PIK3CA gene in the 3q26 amplicon. Consequently, PIK3CA and ALG3 are co-amplified in 89%, 28% and 76% of lung squamous cell carcinoma and breast and ovarian carcinoma, respectively. We found that depletion of ALG3 leads to improper glycan formation, induces ER stress, and reduces proliferation rates in breast cancer cells. We have also used lectin staining to determine the functional consequence of PI3K/AKT-mediated regulation of ALG3 function and the proper folding and surface expression of receptor tyrosine kinases. Current studies are focused on cellular glycomics approaches to investigate changes in the glycan profile, peptido-glycomics, as well as *in vivo* studies. Our findings advance our understanding of the regulation of N-glycosylation by oncogene-driven signaling and its role in cancer progression, and pave the way for exploring future combination strategies targeting PI3K/AKT and protein glycosylation.

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Abstract 1910

Characterizing the Role of TonB7 in *Bacteroides thetaiotaomicron*

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Bacterial microorganisms play an important role in regulating human health and digestion. The Bacteroides are prevalent members of the gut microbial community that are essential for polysaccharide degradation and formation of a healthy gut microbiome. The gram-negative model organism *Bacteroides thetaiotaomicron* (B. theta) relies on specialized membrane proteins known as TonB-Dependent Transporters (TBDTs) to move complex polysaccharides into the cell. As the name suggests, this transport is energized by TonB proteins found in the inner membrane. Previous research began characterization of the 11 identified TonB proteins in B. theta by analyzing the effect of gene deletions on polysaccharide utilization. This research showed that although the TonB7 protein (BT3192) was not present in the membrane fraction of glucose or maltose grown cells, deletion of this gene produced a lag in bacterial growth that was not rescued by complementation. This suggests that removing TonB7 from the genome disrupts some essential process(es) other than TonB interaction with the polysaccharide transporter. To achieve a ΔTonB7 strain without this phenotype, the deletion scheme was redesigned to target two highly conserved portions of the gene that are predicted to interact with the TBDT: the entire C-terminal domain and a beta-sheet within that domain. Strains of B. theta containing each of these deletions will be grown on starch to screen for changes in growth in comparison to wild-type and full gene deletion strains. The resulting strains will also be screened for cell morphology and other growth changes that may suggest a role for this protein or genomic region. We have also expressed and purified the C-terminal domain of TonB7 for structural and functional characterization. Initial trials of *E. coli* recombinant expression resulted in soluble protein. Additional purification steps will be added to obtain protein sufficiently pure for x-ray crystallography trials and assays for TBDT binding. Comparison of the B. theta TonB proteins to those encoded by other Bacteroides species suggests that TonB7 is found only in a limited number of species closely related to B. theta. Therefore, better understanding of the function of TonB7 may reveal a unique niche filled by B. theta and closely related species and will provide a unique approach for manipulating these bacteria.

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Abstract 1934

Effect of carbohydrate binding modules on thermostability, binding, and activity of glycoside hydrolase CelR

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Polysaccharide degradation by glycoside hydrolases (GHs) contribute to the breakdown of plant biomass needed to support the renewable bioeconomy. Understanding the contributions of structural features and domains allows targets for enzyme enhancement. This work focuses on an enzyme from *Acetovibrio thermocellus*, CelR, which belongs to glycoside hydrolase family 9 (GH9). Variants of the catalytic domain with native family 3c carbohydrate module (CBM3c), family 3a CBM (CBM3a), both, and neither CBM were expressed and assayed for thermostability, binding, and activity. Thermostability was measured by digital scanning flourimetry protein melting, binding was measured by pull-down of GFP-tagged inactive variants, and activity was measured using a modified BCA assay to detect reducing sugars. Binding to amorphous cellulose in the form of phosphoric acid swollen cellulose (PASC) was strong for all variants and activity on PASC was higher (10x) with CBM3c. Binding to crystalline cellulose (Avicel) was improved greatly (5x) with CBM3a. CBM3a also improved activity (2–5x) on Avicel, however activity was much higher (10x) with CBM3c. CBM3a gave a slight increase in thermostability (+5°C), but CBM3c had a much greater impact on thermostability (+25°C). Continued work is focused on understanding the reaction mechanism of CelR along with another glycoside hydrolase via time resolved serial crystallography. This work reveals the roles of different CBMs and how they interact with CelR.

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Abstract 1936

Increases in expression of carbohydrate sulfotransferases CHST11 and CHST15 and decline in N-acetylgalactosamine-4-sulfatase (Arylsulfatase B; ARSB) require phospho-p38-MAPK following exposure to SARS-CoV-2 spike protein receptor binding domain in human a

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Sumit Bhattacharyya

Objective: Immunohistochemistry of post-mortem lung tissue from Covid-19 patients with diffuse alveolar damage demonstrated marked increases in chondroitin sulfate and CHST15 and decline in N-acetylgalactosamine-4-sulfatase. Studies were undertaken to identify the mechanisms involved in these effects.

Methods: Human primary small airway epithelial cells (PCS 301–010; ATCC) were cultured and exposed to the SARS-CoV-2 spike protein receptor binding domain (SPRBD; AA: Lys310-Leu560; Amsbio). Expression of the spike protein receptor, angiotensin converting enzyme 2 (ACE2), was enhanced by treatment with Interferon-beta. Promoter activation, DNA-binding, RNA silencing, QPCR, Western blots, ELISAs, and specific enzyme inhibitors were used to elucidate the underlying molecular mechanisms.

Results: Treatment of the cultured cells by the SPRBD led to increased CHST15 and CHST11 expression and decline in ARSB expression. Sulfotransferase activity, total chondroitin sulfate, and sulfated glycosaminoglycan (GAG) content were increased. Phospho-T180/T182-p38-MAPK and phospho-S423/S425-Smad3 were required for the activation of the CHST15 and CHST11 promoters. Inhibition by SB203580, a phospho-p38 MAPK inhibitor, and by SIS3, a Smad3 inhibitor, blocked the CHST15 and CHST11 promoter activation. SB203580 reversed the SPRBD-induced decline in ARSB expression, but SIS3 had no effect on ARSB expression or promoter activation. Phospho-p38 MAPK was shown to reduce retinoblastoma protein (RB) S807/S811 phosphorylation and increase RB S249/T252 phosphorylation. E2F-DNA binding declined following exposure to SPRBD, and SB203580 reversed this effect. This indicates a mechanism by which SPRBD, phospho-p38 MAPK, E2F, and RB can regulate ARSB expression and thereby impact on chondroitin 4-sulfate and dermatan sulfate and molecules that bind to these sulfated GAGs, including Interleukin-8, bone morphogenetic protein-4, galectin-3 and SHP-2 (Src homology region 2-containing protein tyrosine phosphatase 2).

Conclusions: The enzyme ARSB is required for the degradation of chondroitin 4-sulfate and dermatan sulfate, and accumulation of these sulfated GAGs can contribute to lung pathophysiology, as evident in Covid-19. Some effects of the SPRBD may be attributable to unopposed Angiotensin II, when Ang1–7 counter effects are diminished due to binding of ACE2 with the SARS-CoV-2 spike protein and reduced

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production of Ang1–7. Aberrant cell signaling and activation of the phospho-p38 MAPK and Smad3 pathways increase CHST15 and CHST11 production, which can contribute to increased chondroitin sulfate in infected cells. Decline in ARSB may occur as a consequence of effects of phospho-p38 MAPK on RB phosphorylation and E2F1 availability. Decline in ARSB and the resulting impaired degradation of sulfated GAGs have profound consequences on cellular metabolic, signaling, and transcriptional events.

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Abstract 1954

The Activity of GFP-Tagged ST6GalNAc-II Against a Series of Differently Charged PSGL-1 Glycopeptides

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Mucin-type O-glycosylation is initiated by a family of polypeptide N-acetylgalactosaminyltransferases (GalNAc-Ts) that transfers a sugar N-acetylgalactosamine (GalNAc) onto the hydroxyl groups of serine and threonine residue proteins. Studies have shown a correlation between GalNAc-T expression and pathological diseases, like cancer and other diseases. The GalNAc containing Ser and Thr resides can further be elongated by core transferases that add other sugars, while further elongation is terminated through the transfer of a sialic acid onto these glycans via the ST6GalNAc-I&II. Ongoing studies within the Gerken Lab show that flanking charged residues N- and C-terminal to the site of glycosylation may change the glycosylation rates of the GalNAc-Ts and the core elongating transferases. In this study, we used a series of model PSGL-1 glycopeptides to examine the roles of flanking charge residues on the activity of an N-terminal GFP-tagged ST6GalNAc-II transferase. The P-selectin glycoprotein ligand-1 (PSGL-1) is expressed on leukocytes and mediates the rolling of endothelial cells, initiating an inflammatory process. For proper function, PSGL-1 requires a site-specific O-glycan structure, which is a heptasaccharide sidechain containing a Sialyl-Lewis X (SLex) determinant. We observed that the enzyme preferred negatively-charged wild-type glycopeptides over more positive glycopeptides. Our initial hypothesis was that the negative charge of the wild-type PSGL-1 glycopeptide would have reduced ST6GalNAc-II activity, as the early addition of sialic acid on the GalNAc would have terminated SLex glycan elongation. Our findings therefore suggest that other features may play a role in the specificity of GFP-tagged ST6GalNAc-II, which may include the presence of the GFP tag.

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Abstract 1975

Enzymes for chemoenzymatic synthesis and degradation of glycans and glycoconjugates

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Carbohydrate-containing structures on the cell surfaces of human and bacteria play important biological and pathological roles. Enzymes involved in the synthesis and degradation of carbohydrates are directly related to the health and the diseases of human. We have developed a diverse array of chemoenzymatic strategies that combine the flexibility of chemical synthesis with regio- and stereo-specific enzyme-catalyzed reactions for highly efficient synthesis of complex and biologically important carbohydrates and glycoconjugates in preparative and multigram scales. These compounds including glycosphingolipids, glycan probes, human milk oligosaccharides (HMOSs), glycosidase inhibitors, and bacterial surface polysaccharide glycans are important probes for substrate specificity studies of glycosyltransferases and glycoside hydrolases for better understanding their biological roles. The interplay of carbohydrate-active enzymes, their substrates and products, as well as related applications will be presented and discussed.

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Abstract 1977

Motif-dependent interactions with OGlcNAc transferase regulate substrate selection and cellular responses

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Dynamic OGlcNAcylation (O-linked β-N-acetylglucosaminylation) on intracellular proteins plays pivotal roles in numerous biological processes in health and disease. OGlcNAc transferase (OGT) is the sole enzyme to catalyze this modification. Understanding the underlying mechanism of how OGT recognizes thousands of protein substrates without a conserved sequence motif is important for its functional regulation and future biomedical applications. Here we report a series of OGT-binding peptides identified from proteomic peptide phage display (ProP-PD) share a short linear motif (SLiM). X-ray crystallography revealed that the SLiM-containing peptides are bound on a shallow groove located in OGT intervening domain (Int-D), a domain with unique folding and enigmatic function. Structure-guided mutagenesis studies indicated that SLiM-dependent association with Int-D regulates OGT-protein interactions and O-GlcNAcylation of specific substrates. Remarkably, disruption of Int-D binding dysregulates cellular stress responses, protein localization, and cancer cell stemness. Overall, our findings uncover a novel function for Int-D as a facilitator of motif-dependent interaction to coordinate OGT substrate discrimination, elucidating a new secondary binding site for development of OGT-targeting therapeutics.

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Abstract 1985

SARS-CoV-2 infection alters the breast milk glycomic profile

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Breast milk is generally accepted as the perfect source of nutrition for the health and development of infants. It also assists in infant innate and adaptive immunity through many proteins that are decorated with glycans. Examples of these glycoproteins include IgA, IgG, and innate immune lectins. Maternal diet and environmental exposure such as pathogens and pollutants affect human milk composition including its glycoprofile. Despite altered glycosylation can have a consequence on the nursing infant's health and immunity, the current knowledge is still emerging in this area of study. COVID-19 has gained attention in recent years by causing severe morbidity and mortality. Similar to other infectious diseases such as influenza, our lab recently revealed alterations in glycome of plasma and different tissue samples of COVID-19 infected patients. Inspired by these findings, we are interested in disclosing the effect of SARS-CoV-2 on glycosylation of breast milk proteins. Toward this, we performed a large-scale systematic study using our high-throughput lectin microarray analysis technology. We analyzed 132 control samples (breast milk collected pre-COVID) and breast milk from 78 COVID-19 infected mothers. Our data showed there is a 4-fold increase in ∝-2,3 sialic acid on glycoproteins that is associated with SARS-CoV-2 infection in lactating mothers. Lectin pulldown experiments further testified to these findings. Given the significance of ∝-2,3 sialic acid glycan signature in infectious diseases, our finding could provide valuable insight into therapeutic development.

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Abstract 2028

Protein O-GlcNAcylation modulates the secretome of keratinocyte and its paracrine regulation of fibroblast function

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Background: Keratinocytes in the epidermis of skin have a crucial role in regulating the functions of fibroblasts in dermis by secreting pro-inflammatory and pro-fibrotic cytokines via paracrine effects. Abnormal changes of this paracrine effect may contribute to the dysregulated fibrosis in diseases such as scleroderma. Protein O-GlcNAcylation is the addition of the GlcNAc moiety from nucleotide uridine diphosphate-N-acetyl-glucosamine (UDP-GlcNAc) onto serine or threonine residue of cytosolic proteins. This is catalyzed by O-GlcNAc transferase (OGT), and removed by O-GlcNAcase (OGA). Abnormal levels of O-GlcNAcylation of proteins are found in various chronic diseases including diabetes, cardiovascular diseases, cancers and Alzheimer's disease. Objective of this study was to determine the role of protein O-GlcNAcylation in regulating secretion of cytokines by keratinocytes and the consequent impact on fibroblasts function. Methods Both primary keratinocytes and dermal fibroblasts were isolated from the wildtype C57BL/6 mouse and cultured separately *in vitro*. The keratinocytes were treated with a chemical inhibitor to either OGT (OSMI-1) or OGA (Thiamet-G) for 24 hours. At the end of treatment, the inhibitor-containing media was removed and keratinocytes were cultured in fresh media for 48 hours. The keratinocyte-conditioned media (K-CM) was then transferred from the keratinocytes to fibroblasts. After 48-hour culture in K-CM, the fibroblasts were harvested for further analysis of markers for fibrosis. Alternatively, the K-CM was processed for secretome analysis by Mass Spectrometry. The inhibitor-treated donor keratinocytes were harvested for analysis of markers for terminal differentiation, apoptosis, autophagy, and cell senescence. In addition, transcriptomic analyses of the donor keratinocytes by RNA Sequencing were carried out to explore alterations in global transcription. Results 1), Chemical inhibition of OGT significantly impaired the terminal differentiation of keratinocytes, which was conversely enhanced by chemical inhibition of OGA. However, neither treatment had any effect on apoptosis or autophagy of keratinocytes; 2), Transcriptomic analyses by RNA Seq revealed global transcription changes in various signaling pathways; 3), Secretome analysis of K-CM revealed that the levels of some profibrotic cytokines including Connective Tissue Growth Factor and Fibroblast Growth Factor 21, etc. were significantly lower, while the levels of some anti-inflammatory proteins such as Heme Oxygenase-1 were significantly higher, in OSMI-1-treated K-CM; 4), The gene expressions of both Acta2 and Collagen I were significantly downregulated in fibroblasts cultured in OSMI-1-treated K-CM. 5), Culture in OSMI-treated K-CM also induced significant caspase-dependent apoptosis in fibroblasts.

Conclusions These findings demonstrate that inhibition of protein O-GlcNAcylation in keratinocytes alters their production of certain cytokines that regulate activation and turnover of fibroblasts. Protein O-GlcNAcylation may be a novel therapeutic target in treating dysregulated fibrosis in diseases such as scleroderma. We are currently combining secretome analysis with an antibody-based multiplex cytokine assay to identify candidate cytokines that are regulated by protein O-GlcNAcylation in keratinocytes. In addition, we are validating the cell culture findings in an *in vivo* mouse model of skin fibrosis.

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Abstract 2053

Glucose Bioenergetics Regulate Protein O-GlcNAcylation in Human Airway Smooth Muscle Cells

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Lori Mavrakis, Vincent Hascall, Suzy Comhair, Reynold Panettieri, Serpil Erzurum

Rationale: O-linked N-acetylglucosamine (O-GlcNAc) addition to Ser/Thr residues of proteins [O-GlcNAcylation] is regulated by the activities of O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), which catalyze the addition and removal of UDP-GlcNAc on the protein, respectively. O-GlcNAcylation utilizes glucose for UDP-GlcNAc synthesis. Glucose metabolism is complex, being used for energy production as well as for biomolecule synthesis. The level of protein O-GlcNAcylation in asthma has not been described although energy metabolism is altered in asthma and linked to asthma pathophysiology. Here, we investigate protein O-GlcNAcylation in asthma and the impact of glucose energy metabolism on protein O-GlcNAcylation. We hypothesize that glucose metabolism regulates protein O-GlcNAcylation, and inhibition of glucose oxidation will increase glucose availability for UDP-GlcNAc synthesis, leading to overall increased protein O-GlcNAcylation.

Methods: To test this hypothesis, human airway smooth muscle cells (HASMCs) were isolated from lungs of asthmatics ($n = 5$) and non-asthmatic controls ($n = 5$). Cells were assessed at baseline culture and with blockade of mitochondrial glucose oxidation by UK5099, an inhibitor of mitochondria pyruvate carrier 1. Protein O-GlcNAcylation (detected by CTD110.6 antibody), OGT, OGA, and AMP-activated protein kinase proteins (Western Blotting) and cellular bioenergetics (Seahorse) were evaluated at baseline and in response to UK5099 in non-asthmatic and asthmatic HASMCs.

Results: OGT and OGA proteins were similar between asthmatic and non-asthmatic cells at baseline. Despite similar level of protein O-GlcNAcylation, non-asthmatic and asthmatic HASMCs had distinct differences in protein O-GlcNAcylation at baseline. Inhibition of mitochondrial glucose oxidative metabolism in HASMCs with UK5099 (10 μM) increased the protein O-GlcNAcylation in non-asthmatic control cells (1.6 ± 0.5-fold, $p < 0.05$), but not in asthmatic cells (0.9 ± 0.2-fold, $p = 0.34$). OGT and OGA proteins were not affected in response to UK5099 treatment in either non-asthmatic or asthmatic HASMCs. In asthmatic, but not in non-asthmatic cells, UK5099 decreased mitochondrial respiration by $38 \pm 5\%$ ($p < 0.01$), increased glycolytic reserve capacity by $40 \pm 15\%$ ($p = 0.06$), and increased phosphorylation of the intracellular nutrient and energy sensor AMP-activated protein kinase at threonine 172 (1.5 ± 0.1-fold, $p < 0.01$). The greater AMPK phosphorylation occurs upon cell sensing lower energy status, and stimulates pathways for energy (ATP) production while suppressing pathways that utilize ATP.

Conclusion: These data indicate that glucose metabolism and O-GlcNAcylation are linked in HASMC. However, asthmatic HASMC regulation of O-GlcNAcylation and glucose metabolic pathways are different than non-asthmatic cells, suggesting that asthmatic cells have different bioenergetic sensing.

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Abstract 2103

X-Ray Scattering of Carbohydrate Chains in Solution

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Jonathan Monroe, Christopher Berndsen

The large polymer of glucose, starch, is a form of long-term energy storage in plants and is one of the most widely used molecules by humans for nutritional and industrial purposes. Plants periodically degrade stored starch as fuel for metabolism and other metabolic processes. Currently, there is limited information on how starch-degrading enzymes, including amylases, bind to and cleave starch. This project aims to describe the structure of the carbohydrate substrate of amylases, thus allowing us to better describe how amylases bind to and degrade starch. While starch has been described in the solid state, the solution structure, where the amylases function, is less clear. Knowing information about the structure of our substrate, maltodextrin, can lead to information about the functionality of amylases through activity assays, thus allowing us to further understand the function of amylases in humans and plants. The main techniques that we used to study the structure of starch were Small Angle X-Ray Scattering (SAXS) and in silico starch modeling. We compared a variety of potential amylase substrates and model substrates of amylases, including soluble starch and maltodextrin chains of varying lengths. The initial results from our SAXS datasets revealed impurities in our commercially acquired polysaccharides. We then purified maltodextrin samples using ethanol precipitation and liquid chromatography to enrich the samples for the target carbohydrates. We observed that the purification method led to clearer SAXS results compared to SAXS datasets on unpurified maltodextrin. We then used Size Exclusion Chromatography coupled SAXS (SEC-SAXS) and High-Throughput SAXS (HT-SAXS) to assess the structure of purified maltodextrin chains in solution. We then fitted three potential forms of maltodextrin based on the solid state structures ranging from compact to extended, and observed their similarities and differences to our SAXS data. The solid state-based models did not match our SAXS data. We then successfully performed molecular dynamics on the solid-state models to obtain a better fit to our data. Our aim is to further refine our simulated dynamics to fine-tune the fit of these structures. We are further applying this purification pipeline to starch and model starch systems. From our current and future work, we will describe and compare the substrate specificity of amylases leading to a better understanding of plant starch remodeling, which will aid in the development of new uses of starch for humans.

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Abstract 2149

Deleting Predicted ExbB and ExbD Proteins in *B. thetaiotaomicron*

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Rebecca Pollet

Bacteria in the gut microbiome assist in digestion by breaking down large polysaccharides that are otherwise indigestible by the host. *Bacteroides thetaiotaomicron* (B. theta) is an abundant bacteria in the human gut microbiome that is able to degrade a diverse array of polysaccharides by utilizing the proteins encoded by Polysaccharide Utilization Loci (PULs). Key components required for polysaccharide uptake are the TonB-dependent transporter, TonB, ExbD, and ExbB proteins. The ExbD and ExbB inner membrane proteins assist in the movement of polysaccharides by using proton motive force to move TonB and the associated transporter. In B. theta, many ExbD and ExbB homologs have been predicted due to similarities in protein alignments to *E. coli* ExbD and ExbB. In this research, the roles of predicted ExbD's BT2666 and BT2667, and of predicted ExbB BT2668 in polysaccharide uptake are investigated by deleting these genes from B. theta's genome using allelic exchange methods and natural bacterial recombination. Sequencing of plasmids designed to delete BT2667 and BT2668 showed successful formation of the deletion plasmid and candidate B. theta strains with each single deletion have been identified. Sequencing amplifications of the genomic region of interest will provide evidence for a successful gene deletion, allowing for a proper analysis of growth patterns of mutant B. theta strains. Strains with multiple deletions of these genes as well as the predicted ExbD protein BT2666 and TonB protein BT2665 will also allow us to explore the role of the full predicted inner membrane complex and the contribution of each of these components to the overall complex function. For each strain, growth patterns on various polysaccharides and cell morphology will be studied to determine the effect of the deletion and therefore the role of the encoded protein in polysaccharide utilization. This work will significantly expand our understanding of ExbB and ExbD proteins and may provide additional targets for manipulating polysaccharide degradation by the Bacteroides species.

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Abstract 2162

Structural Characterization of TonB Proteins from *Bacteroides thetaiotaomicron*

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Rebecca Pollet

Bacteroides thetaiotaomicron (B. theta) exists in the large intestine and helps break down polysaccharides via polysaccharide utilization loci (PUL). Within these outer membrane complexes, TonB-dependent transporters (TBDT) feature a conserved motif known as the Ton Box. The polysaccharides bind to the TBDT leading to a reaction between the Ton Box and the inner membrane protein TonB. This opens a channel in the TBDT, allowing the polysaccharides to come into the cell. B. theta encodes 11 predicted TonBs that are a part of the larger intermembrane Ton complex allowing for polysaccharide uptake. Our research is focused on the structure and function of TonB2, 3, and 5. To express these proteins, we amplified a plasmid to create a linear pETite backbone including a His-tag and amplified the region encoding for the C-terminus of each TonB protein. We combined the two pieces of DNA and transformed them into *E. coli*. All three protein genes were successfully cloned. They were sent for sequencing to confirm the formation of the plasmid, and TonB2 and 3 were successful. The plasmid was then inserted into Rosetta pLysS cells for protein expression. We initially used affinity chromatography to purify the TonB proteins. We obtained soluble protein for TonB2 and 3 but neither was purified sufficiently. Therefore, we have added an ion exchange step to further purify both of the proteins. For TonB3, ion exchange worked but needs to be repeated to confirm no degradation of the protein occurred. Future work includes adding more steps such as size exclusion chromatography to the purification process and applying these protocols to TonB5. Once we have obtained purified protein, we will use x-ray crystallography to determine the structure of the proteins and test binding to the TonBox of TBDT via ITC, which will help us to understand the interaction between TonB and the TBDT.

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ASBMB

Abstract 2204

Development and Use of Glycan-Binding Monoclonal Antibodies

Jeff Gildersleeve, *NIH/NCI*

Monoclonal antibodies to carbohydrates are useful for many basic research and clinical applications. For example, Unituxin (dinituximab) is an antibody that binds the tumor associated ganglioside GD2 and is FDA approved for treating neuroblastoma. Unfortunately, there are very few high-quality antibodies that target carbohydrates. To help alleviate this problem, we are developing new approaches for anti-glycan antibody discovery and isolation as well as databases with extensive information about existing antibodies. A key tool for these efforts is our glycan microarray, which allows us to rapidly profile affinity and selectivity of anti-glycan monoclonal antibodies. The array contains a diverse collection of O-linked glycans, N-linked glycans, glycosaminoglycans, lipid glycans, glycopeptides, and glycoproteins. Using the microarray, we have developed a variety of new glycan-binding antibodies, including an antibody that selectively binds GalNAca-Tyrosine and a human antibody that selectively binds fully deacetylated PNAG in bacterial biofilms. This presentation will describe the technology and approaches we use to identify new, high-quality monoclonal antibodies as well as their binding properties, structural basis for recognition, and applications.

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Abstract 2212

Rodent Stabilin-2/HARE is a Clearance Receptor for Unfractionated Heparin

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Edward Harris

Background: The clearance of systemic circulatory hyaluronan (HA) is mediated by the scavenger receptor, Stabilin-2. Stabilin-2 is expressed in native tissues as two isoforms; a large 300 kDa monomeric isoform which is encoded by the full-length processed gene and a smaller isoform that is presumably a proteolytically processed product of the large isoform that is 175 kDa in mass. This smaller isoform was initially cloned and characterized in the rodent model (rat) and named the Hyaluronic Acid Receptor for Endocytosis (HARE) around 25 years ago. Thus, the large isoform is named Stabilin-2 and the smaller isoform is named HARE, though they both perform the same function in human tissues. Stabilin-2/HARE are predominantly expressed in the sinusoidal endothelial cells of the liver (LSECs), lymph node, spleen and bone marrow. Their expression in the LSECs which interfaces the blood on one side and the Space of Disse on the other is what makes these receptors optimal for their clearance function. Human Stabilin-2/HARE are better characterized as clearance receptors for numerous blood-borne ligands such as hyaluronan, heparin, chondroitin sulfate, phosphatidylserine, oxidized low density lipoprotein, advanced glycation end-products, Factor VIII and others.

Hypothesis: There has been some controversy in the field as to whether heparin is a ligand for rodent Stabilin-2/HARE and we set to prove our hypothesis that this receptor does bind heparin.

Objective: The objective of this study is to resolve this issue by testing if recombinant Stabilin-2/HARE binds and internalizes unfractionated heparin (UFH). Both Stabilin-2 and HARE were independently expressed in stable Flp-In 293 cell lines and tested for heparin binding and endocytosis using HA as a control.

Results: The results indicate that heparin binding and endocytosis to rat Stabilin-2/HARE is comparable with human Stabilin-2/HARE, though the affinity of UFH may be slightly lower for the rat receptors. During these experiments, it was also discovered that the rat receptors have a faster turnover rate and same affinity for HA than the human receptors and the production of HARE from the Stabilin-2 protein in human recombinant cells is very low compared to what occurs in native rat tissues.

Conclusion: Our conclusions are that the rat receptors are similar to the human protein receptors and that the rodent model is a relevant physiological model for human metabolism and disease regarding Stabilin-2/HARE activity.

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Abstract 2265

Integration of glycan-related disease information and ECM protein-GAG interaction data into the GlyCosmos Portal

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Masae Hosoda, Tamiko Ono, Kiyoko Aoki-Kinoshita

Glycans have been studied to play vital roles in all living organisms, and databases have been developed to collate glycan information for easier access to obtain glycan information. Still, numerous glycan-related information in the scientific literature is not collated in those databases. Moreover, databases with glycan-related information have been developed independently and are scarcely cross-linked or cross-referenced, which makes it hard for the users to find only the glycan information. The GlyCosmos Portal aims to integrate all glycan-related information scattered across multiple databases and literature into one Web portal. Therefore, to enrich the glycan information in the GlyCosmos Portal, we integrated glycan-related disease data from the Alliance of Genome Resources and protein-GAG interaction data from MatrixDB. We obtained these data and related resources from GlyCosmos, FlyGlycoDB, KEGG, and the GlycoGene Database GGDB. Because the data in GlyCosmos is stored using Semantic Web technologies, the curated data was converted to Resource Description Framework (RDF) format based on the Human Disease Ontology, the OBO Relations Ontology, and the MatrixDB identifiers. The RDF data was then integrated into and made browsable under the "Pathways/ Interactions/ Diseases/ Organisms" page in GlyCosmos. Consequently, glycan-related disease data stored in the Alliance of Genome Resources can now be searched in GlyCosmos with gene names, gene IDs, and species names responsible for the related disease names as well as the association types, evidence code names and references. Regarding carbohydrate-protein biomolecular interaction data from MatrixDB, protein-binding gene names, their IDs and species names can be found easily in GlyCosmos. Additionally, each gene has a detailed summarizing gene- and glycan-related information in GlyCosmos.

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Abstract 2268

Mapping the O-GlcNAcylation sites on NOD2 innate immune receptor

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Ophelia Ukaegbu, Natasha Zachara, Catherine Grimes

Nucleotide-binding oligomerization domain containing 2 (NOD2) is an innate immune receptor that recognizes different fragments from bacteria and viruses. It has become prominent since the discovery of three NOD2 polymorphisms conferring a striking 17.1-fold increased risk for developing Crohn's disease, a common inflammatory bowel disease (IBD). While different NOD2 downstream signaling mechanisms have received substantial interest, there is significantly less information about the regulation of NOD2. We reported that NOD2 is a subject for O-GlcNAcylation, a dynamic post-translational modification (PTM) that adds the monosaccharide O-GlcNAc to Ser/Thr residues of proteins to modulate their stability and functions. Here this study aims to investigate the mechanism of this modification on NOD2 and its implication on NOD2 functions, with a goal to enhance our understanding of regulatory system of NOD2. We first utilized the UDP-Glo assay to confirm that NOD2 is O-GlcNAcylated *in vitro*. Based on previously published data, we predicted that a serine residue near the ligand binding site on the C-termini of NOD2, to be a potential O-GlcNAcylation site. To test our hypothesis, we created a cysteine mutant that would covalently capture the sugar O-linked GlcNAc. Biochemical characterization of the mutant showed that it has similar secondary structures but an enhanced expression and stability compared to WT NOD2, which can be potentially used for crystallography. When subjecting this mutant to proteomics analysis, however, we found no modification at this site but two O-GlcNAcylation sites on the CARD domain on the N-termini of NOD2, where NOD2 interacts with other proteins. The proteomics results led us to create a variety of mutations at the new sites, which will be used to study the effect of O-GlcNAcylation on NOD2 structure and function via different biochemical assays (i.e., immunoprecipitation, a dual-luciferase reporter assay, etc.). Our findings will fill in the knowledge gap of NOD2 regulation, further contribute to the overall understanding of NOD2 and potential novel therapeutic modalities.

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ASBMB

Abstract 2295

Synthesis of Immunostimulatory peptidoglycan fragments

Catherine Grimes, *University of Delaware*

Rachel Putnik, Madison Anonick, Hans-Christian Reineicker, Shuyuan Chen

Bacterial cells surround themselves with a peptidoglycan (PG) cell wall, an essential structure that resists changes in osmotic pressure and other environmental insults. The goal of this proposal is to develop a PG fragment library that captures the natural diversity to facilitate the proper innate and adaptive immunity biological studies. PG fragments are used by the innate immune system to correctly recognize and respond to the presence of bacteria. We hypothesize that PG diversity naturally present across the multitude of bacterial species is essential for generating the correct immune response for host defense. Study of these important PG fragments has been hampered by the lack of reproducible, high purity compounds. Currently, researchers are limited to few carbohydrate probes, such as MDP, and even fewer larger fragments. Here we describe the synthesis of three functionalized carbohydrate cores: monosaccharide, disaccharide and peptide dimers, with select amino acids which are highly represented across a variety of bacterial species. The synthetic workflow is modular, builds off our expertise in producing highly pure PG fragments and permits for modification with chemical biology probes at multiple pinpoints on the library members. Using this workflow, the disaccharide components were found to signal differently than monosaccharides using an unbiased genome wide transcriptome analysis to unravel gene expression signatures that define the responses to PG classes in bone marrow derived macrophages. Finally, we probed the innate immune response of the PG through the production of a PG-microarray. This array fixed the PG derivatives in multiple orientations permitting the sweeping of potential receptors across chemical space. The arrays was used to assess the substrate binding preferences of a variety of innate immune receptors indicated in PG recognition. Our data suggest that this library will accurately capture the PG fragment diversity, providing powerful probes for the proposed immunological assays and new tools for the microbial and immunological communities.

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Abstract 2340

Interaction of Glycosaminoglycans with Cathepsin D

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Alzheimer's Disease (AD) is a chronic neurodegenerative disorder and the most common form of dementia, which is characterized as a decline of cognitive abilities. Classically, the extensive neuronal loss in AD is thought to arise due to amyloid plaques and neurofibrillary tau tangles. One of well-known degraders of tau tangles is cathepsin D (catD), which is a lysosomal aspartyl protease. Long time ago, catD was shown to bind to glycosaminoglycans (GAG), a group of natural, highly sulfated polymers that are known to interact with many proteins relevant to AD including Abeta and tau. Yet, how GAGs modulate these proteins, especially catD, has been poorly understood. In fact, lack of molecular understanding on GAG – catD interaction precludes designing and discovering GAG-like molecules that could enhance tau degradation or clearance. Interestingly, contradictory studies on the effect of GAGs on catD are available from the literature. Whereas one report finds GAGs to inhibit catD, another has found a stimulatory role. To resolve some of these quandaries, we performed genetic algorithm-based molecular docking studies to understand GAG recognition by catD. Screening of commercially available GAG sequences, we noted two distinct putative GAG-binding sites on catD, of which one of the sites was the preferred site. Further, the preferred GAG-binding site displayed a strong preference for a few commercially available GAG sequences. Interestingly, the projected in silico affinity of the library of GAG sequences was neither dependent on chain length nor the sulfation density, but on the pattern of sulfation. This implies that certain GAG sequences may be able to recognize catD better, which supports the idea that catD-dependent tau degradation may be modulated by 'optimally sulfated' GAG sequences. These in silico studies are currently being evaluated in *in vitro* studies of catD activation.

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Abstract 2351

Tools for the Detection of Polysaccharide Precursors and Surface Polymers

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Complex capsular and secreted polysaccharides play critical roles in bacterial survival, pathogenesis, and symbiotic interactions with hosts. Importantly, the structure of these surface polymers is often different depending on the specific species of bacteria and can act as a unique fingerprint for sensing specific organisms or targeting them with selective therapeutics. However, these applications require ready access to these polymers for the development of materials that can selectively interact with them. Our research group has focused extensively on developing new methods for reconstructing glycan biosynthesis pathways *in vitro*, using fluorescent polyisoprenoids as easily detectable anchors for key phosphoglycosyl and glycosyl transferases. More recently we have also begun using LC-MS strategies in combination with genetic methods to investigate these pathways in whole lysates. Using information gleaned from these studies we have reconstructed the biosynthetic pathway for an important bacterial polysaccharide from the anaerobic *Bacteroides fragilis* in *E. coli*. We have found that the *B. fragilis* CPSA biosynthesis gene locus alone is not enough for CPSA production and added additional sugar-modifying enzyme encoding genes from *Campylobacter jejuni* and *Vibrio vulnificus* to complete the development of an effective CPSA assembly system. In addition, several changes were made in the gene locus to promote ribosome binding of each gene transcript and enhance glycan production. The impact of CPSA production on *E. coli* growth has been surprising, and our work is currently focused on redesigning *E. coli* for effective glycan production. I will describe some of our efforts in this area including the removal of other glycan assembly systems and a directed evolution approach to improving the growth of *E. coli* without disrupting its ability to produce a foreign glycan.

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Abstract 2357

Phosphoglycosyl transferases: Gatekeepers to glycan and glycoconjugate biosynthesis

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Complex glycoconjugates serve critical functions across domains of life. In bacteria, diverse biomolecules, such as the bacterial peptidoglycan and capsular polysaccharide afford mechanical stability to unicellular organisms in rapidly changing environments and often act as virulence factors. Complex glycolipids, including the O-antigen component of the lipopolysaccharide (LPS) of Gram-negative bacteria, function to mediate interactions amongst cells and propagate deleterious pathogenic processes and, glycoproteins in prokaryotes and eukaryotes are important cell-surface determinants involved in myriad cellular functions. The biosynthesis of a majority of glyconjugates occurs via an en bloc mechanism involving sequential stepwise glycan assembly onto a polyprenol phosphate-linked carrier at cellular membranes including the inner membrane of Gram-negative bacteria and the ER membrane of eukaryotes. This presentation focuses on the initial membrane-committed step of the biosynthetic pathways that is catalyzed by phosphoglycosyl transferases (PGTs). PGTs catalyze the transfer of a phospho-sugar from a soluble nucleoside diphospho-sugar to a membrane-resident polyprenol phosphate. Studies on the PGTs have been hampered because they are integral membrane proteins, and often prove to be recalcitrant to expression, purification, and analysis. However, in recent years exciting new information has been derived on the structures and the mechanisms of PGTs, revealing two unique superfamilies of PGT enzymes that catalyze phosphoglycosyl transfer at the membrane interface. Genome neighborhood analysis shows that these superfamilies, the polytopic PGT (polyPGT) and monotopic PGT (monoPGT), may initiate different pathways within the same organism. Moreover, the same fundamental two-substrate reaction is enacted through two distinct chemical mechanisms with differing modes of catalysis. This presentation highlights the structural and mechanistic divergence between the PGT enzyme superfamilies and how this is reflected in differences in regulation in their varied glycoconjugate biosynthesis pathways.

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Abstract 2375

Antibodies against glycans and their role in human diseases

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Susceptibility to structural valve deterioration is one of the major drawbacks of bioprosthetic heart valves (BHVs). N-glycolylneuraminic acid (Neu5Gc) is an immunogenic dietary-carbohydrate antigen in humans because of inactivation of the gene encoding CMP-N-acetylneuraminic acid hydroxylase (CMAH), and all humans have circulating anti-Neu5Gc antibodies. We hypothesized that interaction of anti-Neu5Gc antibodies with Neu5Gc on BHVs could lead to immune response resulting in valve deterioration through calcification. We demonstrate Neu5Gc in both native calcified human valves as well as in calcified-BHVs, explanted from human patients, by HPLC and immunohistochemistry. Furthermore, anti-Neu5Gc IgGs were purified from native calcified human valves, validated by a glycan microarray. In the Neu5Gc-free Cmah-KO mouse model, anti-Neu5Gc antibodies promoted calcium deposits in subcutaneous implanted BHV discs, both with passive transfer of affinity-purified human anti-Neu5Gc IgGs, and by active-immunization of Cmah-KO mice with Neu5Gc-containing glyconanoparticles. Thus, co-existence of Neu5Gc/anti-Neu5Gc likely mediate BHV structural valve deterioration.

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Abstract 2390

Spatiotemporal changes in brain chondroitin sulfate glycosaminoglycan sulfation patterns across species

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Aarun Hendrickson, Kendra Francis, Shannon Hu, Kathy Cui, Zaman Mirzadeh, Dirk Keene, Michael Schwartz, Jarrad Scarlett

The brain's ability to form neural circuits during neurodevelopment and reorganize neuroconnections in adulthood is driven by changes in the extracellular matrix chondroitin sulfate glycosaminoglycan (CS-GAG) sulfation patterning. The repeating units of CS disaccharides are comprised of glucosamine and N-actylgalatosamine isomers that are non-sulfated (0S-CS), mono-sulfated (4S-CS, 6S-CS) or di-sulfated (2S6S-CS, 4S6S-CS, 2S4S-CS/Dermatan). Of particular interest, the mono-sulfated 6S-CS isomer promotes neurocircuit plasticity and restructuring by forming a soft matrix pallet and altering extracellular protein-glycan binding interactions involved in circuit remodeling. Recent studies have shown that in mice, the abundance of 6S-CS isomer in whole brain isolates steady decrease with natural aging, but whether this age-associated change occurs universally throughout the brain, and whether these changes translate to aging in humans, is currently unknown. To further explore the spatiotemporal changes in brain 6S-CS isomer across species, we analyzed the relative abundance of cortical and hippocampal CS isomers using LC-MS/MS in mice and humans throughout life. Our Preliminary Data using 118 mice [51%(m)/49%(f)] from 10 days to 2 years of age shows the hippocampus exhibits a much greater presence of 6S-CS (40.6 ± 0.9% HIP) at 10 days of age compared to the adjacent somatosensory cortex (20.0 ± 1.4% CTX) that then decline at 14 days (30.5 ± 0.3% HIP; 12.1 ± 0.2% CTX), 21 days (23.4 ± 0.5% HIP; 8.5 ± 0.2% CTX), 28 days (17.7 ± 0.3% HIP; 6.5 ± 0.1% CTX), 3 months (6.9 ± 0.1% HIP; 3.7 ± 0.1% CTX), 6 months (5.9 ± 0.1% HIP; 3.4 ± 0.1% CTX), 12 months (5.0 ± 0.1% HIP; 2.4 ± <0.1% CTX), and 18 months (4.7 ± 0.1% HIP; 2.1 ± <0.1% CTX) of age (mean ± SE; 9–27 mice/group). Surprisingly, we observed an increase in the 6S-CS isomer at 24 months of age (5.0 ± 0.2% HIP; 2.3 ± <0.1% CTX), suggesting an age-associated biphasic remodeling of brain CS-GAGs throughout life. We then analyzed the relative abundance of the 6S-CS isomer in n = 52 human brain tissue samples [ages: 1 wk – 95 years, sex: 52%(m); 48%(f)]. Similar to mice, the human hippocampus exhibited a higher abundance of the 6S-CS isomer following birth (1 wk-1 month of age; 19.3 ± 1.8% HIP) compared to the adjacent inferior temporal cortex (13.2 ± 0.9% CTX) that then declined at ages 4–14 years (12.3 ± 1.2% HIP; 7.4 ± 1.0% CTX), 15–30 years (8.2 ± 1.0% HIP; 6.1 ± 0.7% CTX), 31–45 years (8.3 ± 0.2% HIP; 5.7 ± 0.4% CTX), and 46–60 years (7.7 ± 0.6% HIP; 5.7 ± 0.6% CTX). Also parallel to mice, we observed a surprising increase of the 6S-CS isomer starting at 61–85 years (9.5 ± 0.5% HIP; 6.5 ± 0.6% CTX) and 86–100 years

(8.8 ± 1.2% HIP; 8.6 ± 1.3% CTX) of age. Considering the striking increase in 6S-CS abundance recently identified in the brains of Alzheimer's disease patients, these translational findings suggest that the age-associated increase in brain extracellular matrix 6S-CS isomer in mice and humans reflects a late-stage biphasic remodeling response to natural aging that may also mechanistically underlie with disease-related cognitive disorders associated with aging.

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Abstract 2435

Characterizing human milk oligosaccharide mediated prevention of adverse pregnancy outcome in invasive Group B Streptococcal models of disease

Steven Townsend, *Vanderbilt Universtiy*

Preterm birth affects nearly 10% of all pregnancies in the United States, with 40% of those due, in part, toinfections. Streptococcus agalactiae (Group B Strep, GBS), is the most common perinatal pathogen responsible for these infections. Current therapeutic techniques aimed to ameliorate invasive GBS infections are less than desirable and can result in complications in both the neonate and the mother. To this end, the need for novel therapeutic options is urgent. Human milk oligosaccharides (HMOs), an integral component of human breast milk, have been previously shown to possess antiadhesive and antimicrobial properties. To interrogate these characteristics, we examined HMO-mediated outcomes in both *in vivo* and *ex vivo* models of GBS infection utilizing a murine model of ascending GBS infection, an EpiVaginal™ human organoid tissue model, and *ex vivo* human gestational membranes. Supplementation of HMOs resulted in diminished adverse pregnancy outcomes, decreased GBS adherence to gestational tissues, decreased colonization within the reproductive tract, and reduced in proinflammatory immune responses to GBS infection. Taken together, these results highlight the potential of HMOs as a promising therapeutic intervention in perinatal health.

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Abstract 2463

Structural basis for aggrecan-hyaluronan interactions

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Perineuronal nets (PNNs) are honeycomb-like structures that exclusively surround the body of a subset of neurons in the central nervous system. First presumed to represent a physical blockade to the formation of new synapses, it is now believed that PNNs are dynamic structures that regulate the plasticity of the central nervous system. This specialized neural extracellular matrix is composed of glycosaminoglycans and secreted proteins that interact with one another to form the reticular assemblies. Aggrecan (ACAN) is unique among the currently identified components of PNNs proteins found in PNNs because its expression in the central nervous system coincides almost exclusively with PNNs. Furthermore, the formation of nets is impaired in mice lacking Acan, and they exhibit significant defects in plasticity. Taken together, these results suggest that ACAN plays an essential role in the assembly and function of PNNs. ACAN is a ~2,500 amino acid secreted protein that includes three globular regions named G1, G2, and G3 decorated with multiple O-linked carbohydrates in the regions linking the globular segments. In particular, the N-terminal G1 region interacts with the glycosaminoglycan hyaluronan (HA), which is an essential component of PNNs. As such, it is believed that association between ACAN and HA are a foundational event in the assembly of these reticular structures. However, it remains unclear how HA and ACAN associate. Here, we report the crystal structures of ACAN in the absence and presence of an HA decasaccharide. The G1 region of ACAN includes tandem link domains that associate via an extensive interface to form a single structural unit. The glycosaminoglycan is clamped inside a groove that spans the length of the tandem link domains. Comparison of this complex structure to the unbound form of ACAN suggests that a significant change in the conformation of loops in the tandem link domains is necessary to accommodate the HA decasaccharide. These structural results set the stage for analyzing the contribution of HA-ACAN interactions in the assembly of PNNs.

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Abstract 2468

Regulation of cytokinesis by O-GlcNAcylation

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Cytokinetic abscission is the physical separation of daughter cells that concludes mitosis. Abscission initiation and progression are highly regulated processes. To ensure that the onset of cytokinetic abscission is synchronized with the completion of upstream mitotic events, cells have a cell cycle checkpoint known as the abscission checkpoint. Cells arrest abscission in the presence of mitotic errors such as trapped DNA in the intercellular bridge, misformed nuclear pores, under-replicated DNA, and tension at the intercellular bridge. The mechanism by which cells sense checkpoint triggers, and respond by inhibiting the abscission machinery is not well understood. O-GlcNAcylation is the post-translational modification of Ser/Thr residues with O-linked N-acetylglucosamine. O-GlcNAc flux is driven by two enzymes, O-GlcNAc transferase, OGT, and O-GlcNAcase, OGA. Because OGT and OGA are reported to interact with key regulators of abscission, including Aurora B kinase, we tested whether O-GlcNAcylation is involved in abscission checkpoint signaling. Here we show that the enzymes OGA and OGT are required for maintaining an abscission arrest. Given the overlap in substrate specificity of phosphorylation and O-GlcNAcylation, we are investigating the role of O-GlcNAcylation on known phospho-regulatory sequences in proteins that function in abscission. Using publicly available resources including The O-GlcNAc Database (www.oglcnac.mcw.edu), combined with structure function analyses, we are developing hypotheses for how O-GlcNAcylation may regulate key pathways and proteins that maintain faithful cytokinetic abscission.

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Abstract 2470

Activation and Incorporation of Rare Sugars into Bacterial Surfaces

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Our planet is inhabited by trillions of bacteria that live inside and outside of humans. The "skin," or surface, of bacteria is called the cell envelope, which mediates infection of the host and protects bacteria from host immune defense tactics. While Gram-negative bacteria contain a protective outer membrane layer absent in most Gram-positives, almost all bacteria contain polymers composed of unique monosaccharides that extend from the cell surface. Gram-negative bacteria typically contain lipopolysaccharide (LPS) in the outer leaflet of the outer membrane with attached polysaccharides called O-antigens that help mediate interactions with the environment. O-antigens are composed of repeating oligosaccharides that define particular bacterial serotypes, which distinguishes bacterial strains within a single species. Foundational chemical biology work has contributed to our understanding of eukaryotic cell surface composition. However, we still lack a clear understanding of assembly of bacterial surface glycan polymers that contain prokaryote-specific or "rare" sugars. Here, we describe synthetic and chemoenzymatic methods to construct rare nucleotide sugars to study substrate recognition by bacterial glycosyltransferases that build O-antigens. We identify key regions in sugar substrates that are required for substrate binding and activity, and we use this knowledge to design chemical probes that will be used for the construction of synthetic O-antigens. This work will expand our understanding of cellular mechanisms underlying bacterial polysaccharide synthesis, and will teach us more about the roles that rare sugars play in bacterial cellular interactions.

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Abstract 2487

Polysialic Acid: Chemistry and Biology

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In vertebrates, the ∝2,8 linked polymer of sialic acid, polysialic acid (polySia), is mainly known as a modification of the neural cell adhesion molecule NCAM. PolySia on NCAM can be produced by two polysialyltransferases, ST8SIA2 and ST8SIA4, with overlapping but distinct expression patterns and enzymatic profiles. As a major determinant of brain development and plasticity, abnormal levels of polySia as well as variants of the human ST8SIA2 gene have been linked to mental disorders like schizophrenia. In a clinical study we contributed to the demonstration that altered polySia-NCAM serum levels in schizophrenic patients are associated with negative symptoms, cognitive impairments and structural changes of brain areas involved in the pathophysiology of the disease. Correspondingly, mice with a conventional knockout of St8sia2 display deficits of cognition associated with impaired brain developmentresulting in altered long-range connectivity and a loss of interneurons in the prefrontal cortex. Notably, the cognitive deficits of adult St8sia2-/- mice are aggravated by cannabis exposure during adolescence, which in humans is one of the one most prominent 'second hits' that render a predisposed person more susceptible to developing schizophrenia. By use of conditional knockout mouse models we now started to dissect, how ST8SIA2-deficiency in different neuron types and brain regions translates into impairments of cognition. In contrast to the neurodevelopmental impact of ST8SIA2, loss of ST8SIA4 leads to reduced polySia levels in adult mice resulting in altered synaptic plasticity and selective impairments of learning and memory. Based on recent studies, deficits of synaptic transmission and learning of St8sia4-/- mice can be rescued by targeting the underlying synaptic mechanisms and, possibly, also by therapeutic application of polySia. Furthermore, impaired memory of adult St8sia4-/- mice can be ameliorated by housing in an enriched environment, known to stimulate hippocampal neurogenesis. This finding raises intriguing questions concerning the role of ST8SIA4 for the age-dependent decline of the polySia-positive fraction of newborn neurons in the human hippocampus and for their further reduction under the condition of hippocampal atrophy in Alzheimer's disease.

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ASBMB

Abstract 2501

Glysite™ Scout Glycan Screening Kits for comprehensive detection of glycan expression

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Erika Leonard, August Estabrook

Glycan modifications play critical roles in cell function in both healthy and diseased states from controlling proliferation to modulating the immune response. However, because of the structural complexity of glycan compositions, elucidating the mechanistic details of disease biology remains challenging. Lectins are part of a broader group called glycan-binding proteins that recognize glycan chains and mediate their functionality. Their glycan-specific nature also makes them important tools that can help profile, characterize, and capture complex glycans in biological systems. To explore the complexity of glycan modifications, Vector Laboratories provides Glysite™ Scout Glycan Screening Kits, which are fully integrated kits for the detection of glycan expression in tissue sections. Our curated lectin selection enables the detection of the major glycan motifs including sialylation, fucosylation and galactosylation, for the evaluation of glycan distribution in a target specimen. Here, we established a standard workflow of lectin-integrated tissue immunoassays. Formalin-fixed, paraffin-embedded (FFPE) tissue sections were prepared from a selected panel of normal and cancerous tissues including colon, pancreas, breast, kidney, brain, prostate and tonsil. Our lectin histochemical analysis revealed tissue-dependent glycopatterns, and our result was aligned with morphological changes observed in disease progression. Thus, Glysite™ Scout Glycan Screening Kits are easily plugged into your day-to-day tissue immunoassays that allow comprehensive screening of N- and O-glycan profiles in various tissue sections, enabling discovery of novel glycobiology in your research.

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Abstract 2527

Workflow for the reproducible identification and quantification of O-GlcNAcylation in complex proteomes

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The addition of a single N-Acetylglucosamine (GlcNAc) to serine and threonine residues is a common post-translational modification (PTM) of cytosolic and nuclear proteins. This PTM is regulated by two proteins, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), and it is involved in the regulation of most cellular functions. However, methods for the analysis, identification and, specially, the quantification of this PTM are challenging due to several limitations. First, since O-GlcNAcylated protein levels are low, enrichment of O-GlcNAcylated proteins/peptides is required in order to identify them in complex proteomes; second, the glycosylic bond between the GlcNAc and the amino-acid residue is labile under the standard conditions of fragmentation in the mass spectrometer, resulting in low peptide bond fragmentation, making it difficult or impossible to identify the peptide or the site of modification; and finally, protein quantitation methods across multiple samples involves the use of different types of labels, such as Tandem Mass Tags (TMT), this compounds the difficulty of obtaining high quality fragmentation peptide spectra to reliably identify and quantify the peptide and site of modification. Here, we describe a procedure for the enrichment of O-GlcNAcylated proteins utilizing anti O-GlcNAc antibodies and their identification and semi quantification using TMT across multiple samples. We describe the conditions for EThcD fragmentation on an Orbitrap Fusion Lumos Tribrid mass spectrometer that results in the efficient site identification and quantification of the O-GlcNAcylated peptides. The method has been optimized using a set of synthetic peptides and applied to the analysis of O-GlcNAcylaytion in prostate cancer cells. We expect this workflow and MS parameters will be applicable to a variety of samples.

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Abstract 2562

O-GlcNAcylation regulates nascent protein synthesis in cardiomyocytes through effects on the translational regulator eIF2α

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The regulated and reversible modification of Ser/Thr residues by O-linked N-Acetylglucosamine (O-GlcNAc) is termed O-GlcNAcylation. O-GlcNAc addition is catalyzed by the conserved enzyme O-GlcNAc Transferase (OGT), while O-GlcNAc removal is catalyzed by O-GlcNAcase (OGA). Crucially, O-GlcNAcylation is implicated in nutrient sensing, cell growth and stress adaptation. Despite these significant roles, the precise pathways impacted by O-GlcNAcylation and what key substrate proteins are involved remain incompletely understood. A hallmark of cardiomyocyte hypertrophy is the rapid production of new proteins that then assemble into the growing contractile machinery of the myocyte. A key regulator of protein synthesis, the translation initiation factor eIF2α, undergoes phosphorylation during stress causing a switch from general mRNA translation to the translation of stress-adaptive mRNAs. In the present work we investigated whether altering O-GlcNAcylation affects hypertrophic nascent protein synthesis (NPS) in cardiomyocytes. While we found that NPS was significantly elevated by the agonist phenylephrine, this response was suppressed in the presence of an OGT inhibitor (OSMI-1). Treating cardiomyocytes with the proteasome inhibitor MG132 did not rescue the reduction in NPS suggesting that excess removal of new proteins by the proteasome is not an underlying mechanism. Instead, we found that OGT-inhibited cells had increased phosphorylation of eIF2α. Additionally, we found increased protein levels of the stress adaptive factors Atf4 and Chop, whose mRNA translation is promoted by phosphorylated eIF2α. ISRIB, a small molecule designed to suppress the effects of phospho-eIF2α in altering translation, significantly prevented OSMI-1-induced accumulation of Atf4. Furthermore, we used metabolic labeling of glycans in cells, coupled with alkyne-azide 'click' chemistry and streptavidin pull-down, to show that eIF2α can be O-GlcNAcylated in cardiomyocytes. Taken together, these results highlight a novel role of O-GlcNAcylation on cardiomyocyte protein synthesis through the regulation of eIF2α. Future investigations will characterize the upstream mechanisms that promote eIF2α phosphorylation in OGT-inhibited cells and investigate mechanistic details and implications of eIF2α O-GlcNAcylation.

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Abstract 2625

Human brain sialoglycan ligand for CD33, a microglial inhibitory Siglec implicated in Alzheimer's disease

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Ryan Porell, Steve Fernandes, Eila Maenpaa, T. August Li, Kazuhiro Aoki, Benjamin Orsburn, Russell Matthews, Ronald Schnaar

Alzheimer's disease (AD) is characterized by accumulation of misfolded proteins. Genetic studies implicate microglia, brain-resident phagocytic immune cells, in AD pathogenesis. As positive effectors, microglia clear toxic proteins, whereas as negative effectors, they release proinflammatory mediators. An imbalance of these functions contributes to AD progression. Polymorphisms of human CD33, an inhibitory microglial receptor, are linked to AD susceptibility; higher CD33 expression correlates with increased AD risk. CD33, also called Siglec-3, is a member of the sialic acid–binding immunoglobulin-type lectin (Siglec) family of immune regulatory receptors. Siglec-mediated inhibition is initiated by binding to complementary sialoglycan ligands in the tissue environment. Here, we identify a single sialoglycoprotein in human cerebral cortex that binds CD33 as well as Siglec-8, the most abundant Siglec on human microglia. The ligand, which we term receptor protein tyrosine phosphatase zeta (RPTPζ)S3L, is composed of sialylated keratan sulfate chains carried on a minor isoform/glycoform of RPTPζ (phosphacan) and is found in the extracellular milieu of the human brain parenchyma. Brains from human AD donors had twofold higher levels of $RPTP\zeta^{S3L}$ than age-matched control donors, raising the possibility that $RPTP\zeta^{S3L}$ overexpression limits misfolded protein clearance contributing to AD pathology. Mice express the same structure, a sialylated keratan sulfate RPTPζ isoform, that binds mouse Siglec-F and crossreacts with human CD33 and Siglec-8. Brains from mice engineered to lack RPTPζ, the sialyltransferase St3gal4, or the keratan sulfate sulfotransferase Chst1 lacked Siglec binding, establishing the ligand structure. The unique CD33 and Siglec-8 ligand, $RPTP\zeta^{S3L}$, may contribute to AD progression.

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Abstract 2734

Chemoenzymatic synthesis of mucin O-glycans for Functional Glycomics

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Decorating over 80% of secretory and cell surface proteins, mucin O-glycans (or named O-GalNAc glycans) represent a major component of the mammalian glycocalyx and are involved in various biological processes via glycan-protein interactions. The structural alteration of mucin O-glycans during physiological and pathological processes has been frequently observed and used as biomedical targets. Structurally defined mucin O-glycans are thus essential for functional studies but synthetic challenges and their inherent structural diversity and complexity have limited access to these compounds. To streamline the synthesis of mucin glycans, we developed a convergent chemical strategy to synthesize all 8 mucin O-glycan core structures from a single versatile precursor. Multiple glycosyltransferase modules were then precisely programmed to generate nearly 100 desired mucin glycan structures. These glycans were then fabricated into mucin glycan microarrays to study the recognition by O-glycan-specific glycan-binding proteins (GBPs), revealing new interaction details to improve the clinical application of these GBPs. Microarray assay of human serum antibodies revealed disparate bindings to individual mucin glycan structures and identified potential targets for further cancer biomarker discovery.

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Topic Category Lipids and Membranes

Abstract 143

Probe real-time interactions between lipids and membrane proteins in quasi-native membrane environments

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Qiu-Xing Jiang

Membrane proteins like ion channels function in lipid environments. While bulk lipids provide a supporting architecture for these proteins, certain specific lipids play important signaling roles by modulating protein activity. The allosteric modulation via lipid-binding sites often occur through direct interactions with membrane proteins without second messenger pathways. Phosphoinositides are such a type of signal lipids, and have been implicated in interactions with a diverse range of membrane proteins and mediate or regulate a variety of cellular functions. To understand how these lipids regulate protein functions, it is necessary to detect and monitor real-time physical interactions between proteins and lipids with sufficient time resolution, and identify binding sites on the proteins. However, detection of real-time protein-lipid interactions has been difficult, especially in a native environment, because only a small fraction of lipids are tightly bound to the target proteins while the bulk ones are freely diffusive and contribute significant background noise. The purpose of this study is to develop an experimental method that is capable of detecting real-time protein-lipid interactions directly in a lipid membrane. The strategy utilizes luminescence energy transfer (LRT) to locally illuminate lipids around the target protein without interference from the background noise of unbound lipid molecules. The approach is tested on an inward rectifier potassium (Kir)channel for its direct interactions with phosphoinositide lipids. Experimental data show that our new method can reliably detect phosphoinositide binding to the Kir proteins through specific binding sites, and register almost no signal when the binding sites are abolished via site-specific mutagenesis. Our approach thus provides a valuable strategy for probing protein-lipid interactions in a near-native lipid environment.

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Abstract 151

Direct interactions between picornavirus 3CD protein and phosphoinositide lipids found in replication organelles

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Dennis Winston, Jie Yu

Positive-strand RNA viruses disrupt host membrane systems and reconstitute these materials into so-called replication organelles, which likely serve as sites for virus replication and genome packaging. In picornaviruses, these new membranous systems are enriched in phosphoinositide lipids, including phosphoinositide-4-phosphate (PI4P). The 3CD polyprotein is central to membrane biogenesis, as it was previously shown that ectopic expression of 3CD leads to PI4P induction and redistribution, a phenotype similar to that of poliovirus-infected cells. Here, we have shown direct interaction between poliovirus 3CD and PI4P using nuclear magnetic resonance (NMR) spectroscopy, especially providing atomic-level insights into PI4P interaction with both the 3C protease and 3D polymerase derived domains of 3CD. Our studies using paramagnetically-labeled nanodiscs demonstrated that 3C interacts with the membrane through its positively charged N-terminal helix, which also interacts with genome RNA sequences important for coordinating replication and translation processes. Extension of the C-terminus of 3C increases its affinity to PI4P-containing membranes, suggesting a means by which interactions may change between 3CD and its processed counterparts. Adjustments to virus protein-membrane interactions may be an important component of the switch from replication to packaging modes on these membrane systems.

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Abstract 157

RUSH in rafts: membrane microdomains across the secretory trafficking

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How are membrane proteins sorted to their specific cellular locations? Although significant advances have identified a variety of specific motifs responsible for cargo integration into coat/adapter-mediated sorting, such motifs are only present on a small subset of membrane proteins. Thus, the determinants of precise sub-cellular distribution are not known for the majority of membrane proteins. One potential mechanism for organizing membrane protein traffic is sorting by membrane microdomains known as lipid rafts. Such domains are small, dynamic clusters of preferentially interacting lipids and proteins that have been widely implicated in signaling at the plasma membrane (PM) but are likely also present in various endomembranes. Our lab has recently defined the structural determinants of preferential protein partitioning into these ordered membrane domains and shown sorting and trafficking of membrane proteins can be directed by their affinity for a particular membrane environment. To directly assess the role of membrane microdomains in the secretory pathway, we have taken advantage of a robust tool for synchronized protein traffic, known as RUSH (Retention Using Selective Hooks). Here, tagged proteins are retained in specific organelles by a resident "hook," from which they can be quickly released upon introduction of biotin, allowing direct and quantitative analysis of trafficking rates and destinations. We applied this system to a library of transmembrane domain (TMD) constructs to evaluate the role of raft affinity in secretory traffic, and the machinery involved therein. We find that while TMD-encoded raft affinity is fully sufficient for PM sorting, it does not confer rapid exit from the endoplasmic reticulum (ER), which instead requires specific cytosolic motifs to be recognized and sorted by COPII machinery. We find that raft-preferring proteins preferentially associate with specific subsets of ER-exit sites, defined by specific Sec24 isoforms. Moreover, we find that Golgi exit rates are highly raft-dependent, with raft preferring proteins exiting ~2.5-fold faster than mutants with perturbed raft affinity. We rationalize these observations with a kinetic model of trafficking through the secretory pathway. These observations highlight a central role for lipid rafts in sorting in the secretory pathway and establish intrinsic features for raft-mediated cellular trafficking from Golgi. The proposed model helps to understand how TMD proteins migrate from ER to their final post-Golgi destination.

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ASBMB

Abstract 1208

Structure determination of moss PpCesA5 cellulose synthase trimer

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Plant membrane proteins called cellulose synthases (CesAs) make cellulose, the most abundant plant polymer in the world found in plant cell walls. The cryo-EM structure of poplar PttCesA8 trimer has revealed structural insights, but an evolutionary context of CesA structure in other plant species is needed. The aim of my research proposal is to understand the role of plant CesA trimer oligomerization in functional cellulose synthesis and assembly. To approach this aim, I seek to characterize the structure of an early plant moss PpCesA5 in detergent and nanodiscs using SAXS/SANS and cryo-Electron Microscopy (cryo-EM). This structural analysis will provide information on residue-level oligomer interactions and global conformation of moss PpCesA5 trimers. Thus far, PpCesA5 has been successfully expressed in Sf9 cells. Current work involves optimizing the purification of higher order PpCesA5 oligomerization to capture this protein as a trimer and a hexamer of trimers. Future work involves an evolutionary structure comparison with poplar PttCesA8 and cotton GhCesA7 for insights on domains involved in oligomerization and function. This work reveals the importance of structural techniques such as cryo-EM to advance future work in biofuels and new biomaterials.

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Abstract 1234

Untargeted Lipidomics in U87-MG Glioma Cells

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Isocitrate dehydrogenase 1 (IDH1) catalyzes the NADP +-dependent conversion of isocitrate to α-ketoglutarate (α-KG) in the cytosol and peroxisomes. This reaction yields the primary source of NADPH in peroxisomes, which are intracellular organelles found in virtually all eukaryotic cells. Peroxisomes are involved in β-oxidation of very long chain fatty acids, α-oxidation of phytanic acid, degradation of H2O2, and biosynthesis of ether lipids. Mutations in IDH1 drive a variety of cancers, most notably gliomas and glioblastoma, and lead to production of D-2-hydroxyglutarate, an oncometabolite, and also prevent its normal activity, leading to decreased α-KG and NADPH. Peroxisomes are a crucial organelle involved in lipid processing reactions which require NADPH. Because ether lipid biosynthesis is dependent on IDH1-derived NADPH, we hypothesized that cells expressing mutant IDH1 have dysregulated lipid levels due to NADPH deficiency. Using U87-MG glioma cells, we extracted lipids using the Bligh and Dyer liquid-liquid method with chloroform/methanol separation, and analyzed fractions using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Fractions were separated on a C18 column (reverse phase separation) or a HILIC column (hydrophilic separation) using liquid chromatography, and then analyzed on the Bruker Impact II UHPLC-QTOF instrument. Data were converted to mzXML files and uploaded to two widely used metabolomic and lipidomic databases. We were able to identify known lipid standards using our optimized reagent system and detect differences among mutant and wild type IDH1. The optimized system will allow us to detect with high sensitivity the different levels of lipids between IDH1 mutant cells to predict new pathways affected by mutant IDH1-driven tumors.

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Abstract 1308

Direct determination of membrane protein-lipid organization from customizable bilayer and its application to synaptic vesicle fusion

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Kallol Gupta

Multimeric organization of membrane proteins (MPs) and lipids at the cell membrane is fundamental to all living organisms. To study these organizations, we have developed a novel tunable *in-vitro* nativeMS platform that allows direct detection of the organization of membrane proteins and bound lipids from customizable lipid membranes. Both the lipid composition, as well as specific membrane properties (such as curvature, tension, fluidity) of these bilayers can be precisely customized to mimic a target physiological membrane. Subsequent direct nativeMS and MS/MS analysis of these intact lipid membranes can yield the oligomeric states of the embedded membrane proteins, identify bound lipids, and determine the stoichiometry of the bound lipids. Together, this can further quantitatively report how specific membrane composition and biophysical properties, such as lateral pressure, curvature, and fluidity, modulate the functional organization of MPs. Subsequently, we have applied this to synaptic vesicle (SV) proteins where higher order molecular organization, including protein-lipid clustering has been proposed to play a major role in regulating neurotransmitter release. We show how specific lipid binding regulates the oligomeric organization of synaptic vesicle (SV) fusion protein VAMP2 and regulates the speed of vesicle fusion. Our data quantitatively shows that the binding of VAMP2 to phosphatidylcholine (PC) and cholesterol de-oligomerizes the protein into a monomeric state in the native synaptic vesicle bilayer. Subsequently, by combining nativeMS-based results with fluorescence-based fusion assay we show that specific binding of PC speeds up the rate of vesicle fusion by 40%. Finally, using a series of customized lipid membranes that mimic different organellar membranes along the trafficking route of VAMP2, such as ER, Golgi, and Plasma membrane, our data show how VAMP2 binds to different lipids in different organellar membranes encountered during its trafficking path. Surprisingly, we observed that VAMP2 can simultaneously bind to PIP2 in the plasma membrane, while still integrated within the SV bilayer. This serendipitous observation of VAMP2's ability to simultaneously bind to two distinctly different organellar membranes opens a new mechanistic possibility of how lipid binding regulates SV-fusion. Coupling this lipid bilayer nativeMS data with functional assays, we will provide proof and mechanistic insight into this hypothesis. Together, the work presents a quantitative platform to directly study molecular organization of membrane proteins and bound lipids directly from intact lipid membrane and delineate how specific membrane properties regulate functional assemblies of membrane proteins.

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Abstract 1313

Sphingosine-1-phosphate signaling regulates intracellular complement activation to induce cancer metastasis

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Besim Ogretmen

Cancer metastasis is the leading cause of all cancer deaths worldwide. Currently, there is no reliable therapeutic strategy to prevent metastasis. Specific crosstalk mechanisms between sphingolipid metabolic signaling and other cellular effector systems to induce tumor metastasis remains obscure. Here, we demonstrate that pro-survival lipid sphingosine-1-phosphate (S1P) signaling via S1P receptor 1 (S1PR1) activates intracellular complement component 3 (C3) to stimulate cancer cell migration and invasion by inducing NLR family pyrin domain containing 3 (NLRP3) inflammasome formation. Using molecular, pharmacologic, and genetic techniques, we showed that S1P/S1PR1 signaling via AKT phosphorylation, regulates cathepsin L (CTSL) to mediate intracellular C3 complement activation in various cancer cell lines. Through co-immunoprecipitation and proteomics analysis, we identified a novel protein, PPIL1 (Peptidylprolyl Isomerase Like 1) which binds C3 (forming PPIL1-C3 complex) to induce tumor metastasis via NLRP3 inflammasome activation. Furthermore, targeting the S1P-C3-PPIL1 axis mitigated the pro-inflammation and cancer cell migration for limiting tumor metastasis. Also, genetic loss of complement signaling components inhibited lung colonization and metastasis of syngeneic melanoma tumors *in vivo*. In summary, these data help advance our understanding of metastatic cancer metabolism and signaling in the tumor microenvironment while providing insight into developing better innovative treatments to limit tumor metastasis.

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ASBMB

Abstract 1314

Measuring Juxtamembrane Disulfide Bond Formation Using the ToxR Assay

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Katherine Koestler, Simon Sheppard

Mucins are highly glycosylated secreted and transmembrane proteins that form a protective mucous barrier in the epithelial layer of multicellular organisms. The membrane-bound mucins, which includes MUC1, MUC4 and MUC16, localize in the apical surface of epithelial cells. Transmembrane mucins are characterized by an extracellular domain, a single-span transmembrane domain, and a cytoplasmic tail. Mucin 1 (MUC1) is the most extensively studied transmembrane mucin, primarily because of its role in breast cancer. In these cells, MUC1 dimers are trafficked to the nucleus where it associates with a transcription factor that regulates a tumor suppressor gene. Studies have also shown that a cytosolic membrane proximal cysteine-glutamine-cysteine (CQC) motif is essential for the dimerization of MUC1. Mucin 16 (MUC16) is the largest of the transmembrane mucins, and its over-expression is associated with poor prognosis in patients with pancreatic, breast and non-small cell lung cancer. Similarly to MUC1, MUC16 has been shown to translocate to the nucleus, suggesting a potential role in gene regulation. The nuclear trafficking of MUC16 is not as well understood as that of MUC1-C. However, MUC16-C contains a pair of cysteine residues (CLIC) near the cytosolic juxtamembrane domain, comparable to the CQC motif in the juxtamembrane of MUC1, suggesting a possible dimerization and nuclear trafficking mechanism similar to that of MUC1. The ToxR assay has been widely used to study the dimerization of transmembrane domains. The assay uses beta-galactosidase activity as a reporter of dimerization in bacterial membranes. We took advantage that the ToxR assay places the C-terminal end of the transmembrane domain of interest in the oxidizing environment of the bacterial periplasm. Thus, we have extended the use of the assay to study dimerization driven by the membrane-proximal CQC and CLIC motifs in MUC1 and MUC16, respectively. The placement of these motifs in the periplasm allows the formation of disulfide bonds between the C-terminal cysteine residues, as it is believed to occur in cancer cells. Our results indicate that the pair of cysteines in both MUC1 and MUC16 contribute to their dimerization, but to a different extent. Whereas dimerization of MUC1 is primarily dependent on the formation of disulfide bonds, the dimerization of MUC16 may involve sequence specific interactions in the transmembrane domain.

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Abstract 1323

Relationship of Omega-3 and Omega-6 polyunsaturated fatty acids levels in Red blood cells, ROS, and hemolysis in sickle cell disease

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the beta-globin gene that leads to the production of hemoglobin S(HbS) to polymerize when deoxygenated, resulting in the characteristic "sickle" shape of red blood cells (RBCs), hemolysis, and multiple complications, including severe acute and chronic pain. Current therapies for pain in SCD are mainly opioid drugs, and thus the need for safe non opioid prophylactic treatments capable of attenuating pain is critical. Excessive ROS in RBCs can trigger a cascade of oxidative reactions, including altering membrane lipids. Although pain intensity is correlated with intravascular hemolysis and oxidative stress in red blood cells, there is a gap in understanding the molecular mechanisms for pain in SCD. Previous studies have shown that a high dietary intake of omega-3 fatty acids is negatively associated with the incidence of pain severity in SCD. However, the protective mechanism is elusive. Mitochondrial retention and elevated ROS have been previously shown in a high percentage of RBCs in sickle cell disease patients. We hypothesized that altered lipid composition in SCD RBCs due to excessive mitochondrial ROS promotes early destruction of RBCs and release of molecules into plasma and extracellular space that can sensitize nociceptor pain signaling ion channel proteins.

Methods: To investigate the relationship between Omega-3 and Omega-6 PUFA levels and ROS in SCD, we have utilized a mouse model of SCD and SCD patients' blood samples. We evaluated the percentage of omega-3 fatty acids and omega-6 fatty acids in red blood cells (RBC), RBC phospholipids, and plasma by GC/MS and the molecular forms of PUFA in mice and humans with SCD.

Results: The present studies demonstrated that the reduction of omega-3 PUFA in SCD mice and patients positively correlated with the levels of hemolysis, ROS, and pain markers. The RBC DHA levels were 62% lower in SCD patients than control patients. Importantly, the RBC DHA phospholipids were significantly reduced by 78% in SCD patients as compared to control patients. Interestingly, compared with control patients, the SCD patients resulted in a larger increase in RBC ARA by 37% and RBC phospholipids ARA by 125%. Similar trend was observed in preclinical; RBC DHA levels were modulated for SCD mice. In this preliminary study, we used the SCD mice has been used extensively in the recent years to study the pathogenesis and to investigate the potential therapeutic interventions. We found that DHA levels in the RBC are deficient by 72% in SCD mice as compared to control mice. We also measured RBC phospholipid DHA in SCD mice, the levels of RBC phospholipid DHA were lower by 56% in SCD mice than in control mice.

Conclusion: This preliminary data indicates that in addition to omega-3 PUFA, an abnormal increase of omega-6 PUFA arachidonic acid (pro-inflammatory) in SCD mice and patients positively correlates with pain severity. These results indicate that the red blood cell membranes of RBCs of SCD have an imbalance fatty acid composition characterized by a high ratio of pro-inflammatory Omega-6 arachidonic acid (ARA) to anti-inflammatory omega-3 fatty acids of EPA and DHA. These results, therefore, have the potential targets to reduce pain episodes. These results suggest that developing novel RBC targeting PUFA to ease pain in SCD is critical. This novel strategy would open the possibilities of preventive approaches for pain in SCD and other similar severe pain conditions. Improving RBC membrane lipids through effective targeted supplementation of fatty acids would be a novel strategy, profoundly altering the disease course.

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Abstract 1349

Convergent evolution of bacterial ceramide synthesis

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Bacteria synthesize a variety of sphingolipids with diverse physiological functions. Despite their roles in mediating host inflammation, cellular differentiation, and protection from environmental stress, their biosynthetic pathway remained undefined since several essential eukaryotic ceramide synthesis enzymes have no clear bacterial homologue. Using genetic and biochemical approaches, we identified the complete pathway for bacterial ceramide synthesis. Bioinformatic and phylogenetic analyses revealed the presence of these genes in a broad range of bacterial taxa and led to our discovery of the first Gram-positive species to produce ceramides. Biochemical experiments with purified proteins support a model in which the bacterial synthetic pathway operates in a different order than in eukaryotes. Furthermore, phylogenetic analyses are consistent with the convergent evolution of the bacterial and eukaryotic ceramide pathways. Current work is being done to elucidate the species-specific physiological roles of bacterial sphingolipids.

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Abstract 1353

Cell membranes sustain phospholipid imbalance via cholesterol asymmetry

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Plasma membranes are cellular interfaces, insulating from noxious agents and conducting the flow of nutrients and information. These functions are facilitated by a diverse array of lipids, nearly all of which are distributed asymmetrically between the two bilayer leaflets. The details, mechanisms, and functional roles of this compositional asymmetry are central to the physiology of mammalian membranes. Essentially all previous models of biomembrane structure and function have rested upon the implicit assumption that the two membrane leaflets have similar total phospholipid abundances. Here we show that this widely held assumption is generally invalid and for mammalian plasma membranes, dramatically incorrect. Using quantitative lipidomics, we discovered that human erythrocyte plasma membranes have almost two-fold more phospholipids in their cytoplasmic leaflets. We demonstrate that this phospholipid imbalance is enabled by highly asymmetric interleaflet distribution of cholesterol, which can rapidly redistribute to equilibrate its chemical potential. Thus interleaflet phospholipid abundance, lipid interactions, and mechanical constraints combine to maintain cholesterol's ultimate enrichment in the exoplasmic leaflet. The resulting lipid distributions impart novel functional properties to plasma membranes, including a resting tension in the inner leaflet and extremely low permeability of the outer leaflet. This previously overlooked aspect of lipid asymmetry in the mammalian plasma membrane represents an evolution of existing models of membrane structure and physiology.

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Abstract 1367

Dissecting mechanisms of liquid-crystalline lipid droplet biogenesis

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Lipid droplets (LDs) are reservoirs for triglycerides (TGs) and sterol-esters (SEs), but how these lipids are organized within LDs and influence its proteome remains unclear. Using in situ cryo-electron tomography, we show that glucose restriction triggers lipid phase transitions within LDs generating liquid-crystalline lattices inside them. Mechanistically, this requires TG lipolysis, which decreases TG:SE ratio in LD, thereupon promoting SE transition to a liquid-crystalline phase. Molecular dynamics simulations reveal TG depletion promotes spontaneous TG and SE de-mixing in LDs, additionally altering the lipid packing of the phospholipid monolayer surface. Fluorescence imaging and proteomics further reveal that liquid-crystalline phases in LDs are associated with selective remodeling of the LD proteome. Some canonical LD proteins including Erg6 re-localize to the ER network, whereas others remain LD-associated. Model peptide LiveDrop also redistributes from LDs to the ER, suggesting liquid-crystalline-phases influence ER-LD inter-organelle transport. Our data suggests glucose restriction drives TG mobilization, which alters the phase properties of LD lipids and selectively remodels the LD proteome.

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Abstract 1376

The carboxy terminus of oleate hydratase forms a membrane anchor that permits fatty acid acquisition from membranes

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Oleate hydratase (OhyA) is a fatty acid (FA) modifying flavoenzyme that adds water across the 9-cis double bond of oleic acid and other unsaturated FA to make 10-hydroxy FA with immunomodulatory properties. The major pathogen *Staphylococcus aureus* has an ohyA gene and an ohyA knockout strain has severely compromised virulence, indicating OhyA is an important factor in establishing infection. OhyA is made up of three functional domains – FA and flavin adenine dinucleotide binding lobes, and a carboxy terminus of unknown function. Detailed structural information is available for how the lobes collaborate to catalyze the water addition chemistry, but the process by which OhyA solves the topological problem of accessing its insoluble substrate is unknown. Here, we identify the OhyA carboxy terminus as a membrane binding anchor domain that associates with the phosphatidylglycerol bilayer and define the structural transitions that govern membrane binding. The OhyA carboxy terminus consists of three amphipathic α-helices. Using surface plasmon resonance, sub micromolar affinity was observed for OhyA binding the phosphatidylglycerol surface. A synthesized peptide corresponding to this domain also has low micromolar affinity for phosphatidylglycerol. Circular dichroism spectroscopy revealed an increase in α-helicity upon interacting with phosphatidylglycerol liposome, and NMR spectroscopy showed the conformational change orients the positively charged amino acids with the phosphate layer, supporting the idea that the carboxy terminal domain is responsible for membrane association. Cryo electron microscopy shows that the OhyA carboxy terminal domain is dynamic in solution and adopts a single helical conformation in the presence of phosphatidylglycerol that matches the conformation observed in the OhyA crystal structures, supporting the structural changes observed in the peptide by NMR. Molecular dynamics simulations and biochemical analysis of site-directed mutants in the carboxy terminal domain provide a conceptual framework for how OhyA uses the amphipathic helices in its carboxy terminus to bind to the membrane surface and access the FA substrate.

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Abstract 1404

The Effects of Artificial Lipid Environments on Diacylglycerol O-Acyltransferase 2 (DGAT2) Function

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Triglycerides (TGs) are the main chemical form of long-term energy storage and are stored intracellularly in lipid droplets (LDs). TG accumulation is linked to various diseases including cancer, diabetes, cardiomyopathy, and obesity. LDs are generated in the endoplasmic reticulum (ER), where most of the enzymes involved in TG synthesis are located. This includes Diacylglycerol O-Acyltransferase 2 (DGAT2), a monotopic membrane protein that catalyzes the last and only committed step of TG biosynthesis. Specifically, DGAT2 catalyzes the formation of an ester linkage between a fatty acyl-coA and 1,2-diacylglycerol (DAG). DGAT2 catalyzes TG synthesis not only on the cytoplasmic face of the ER, but also on the surface of LDs, when fatty acids are in excess. Alternatively, DGAT2 can utilize pro-apoptotic ceramide as a substrate, instead of DAG, to generate 1-O-acylceramide, which has been implicated in causing cancer cells to become resistant to chemotherapy. Given DGAT2's prominent roles, it serves as a strong candidate for therapeutic targeting. Although we have a general idea about the function and localization of DGAT2, little is known about its structure, the residues that facilitate selective substrate binding (e.g. DAG vs ceramide), and if catalytic activity is altered by diverse membrane environments (e.g. ER lipid bilayers vs. LD lipid monolayers). In this study, we address some of these questions biochemically, using purified human DGAT2 and/or a thermophilic DGAT2 homolog from Chaetomium thermophilum (Ct. DGAT2). We established protocols to detergent solubilize and purify recombinant human DGAT2 and Ct. DGAT2. In addition, we developed enzymatic assays using both thin-layer chromatography (TLC) and liquid chromatography-mass spectrometry (LC/MS) and confirmed purified Ct. DGAT2 is catalytically active. Then, we reconstituted the purified enzyme into various membrane environments, such as liposomes and artificial lipid droplets to mimic ER and LD membranes, respectively. We compared Ct. DGAT2 activity in these environments versus in bovine serum albumin (BSA), the canonical lipid complexing agent used in activity assays. We observed greater TG formation by Ct. DGAT2 in these artificial membrane environments versus BSA, which reaffirms our hypothesis that these artificial membranes might be an optimum representation of physiological conditions to measure DGAT2 activity. For future studies, we hope to study DGAT2 kinetics in these diverse membrane environments. Additionally, we plan to study the structure of DGAT2 and identify key residues necessary for catalytic activity, and substrate specificity. Our hope is that in the future, this research will pave the way for the development and classification of more potent DGAT2 inhibitors, with optimized inhibition in LDs and

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ER membranes, that can improve treatment efficacy for cancer, and other diseases associated with TG accumulation.

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Abstract 1432

Structural determination and internalization dynamics of human Flower isoforms

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Human Flower (hFWE) isoforms hFWE1-4 are putative transmembrane (TM) proteins that reportedly mediate fitness-comparisons during cell competition through extracellular display of their C-terminal tails. Isoform topology, subcellular localization and duration of plasma membrane presentation are essential to this function. However, disagreement exists regarding the structure of orthologous fly and mouse FWEs, and there is no experimental evidence for hFWE isoform subcellular localization or membrane structure. Here, we used AlphaFold and subsequent molecular dynamics-based structural predictions to construct epitope-tagged hFWE3 and hFWE4, the most abundant human isoforms, for experimental determination of their structure and internalization dynamics in HEK293 cells. Colocalization of C-terminal hFWE-EGFP fusion proteins with mCherry chimeras targeting various subcellular compartments revealed that hFWE3 resides in the endoplasmic reticulum (ER), while hFWE4 partially colocalizes with Rab4, Rab5 and Rab11 positive vesicles as well as plasma membrane. Using live cell surface staining, genetically encoded anti-epitope scFV "Frankenbodies," and fluorescence protease protection assays, we showed that hFWE4 positions both N- and C-terminal tails and a loop motif between the second and third TM segments within the cytosol, while small (1-7aa) motifs between the first and second and the third and fourth TM segments are located intracellularly. These techniques also demonstrated that on the ER membrane, hFWE3 similarly positions N- and C- terminal tails into the cytosol, while the loop between its two TM segments is exposed within the ER lumen. Pulse-chase internalization assays showed that the majority of surface bound hFWE4 is internalized after only five minutes of 37°C chase, and proceeds via a mechanism dependent on Dynamin1 activity and the presence of functional AP-2 binding motifs within its N- and C- terminal tails. Our data support a model in which hFWE4, but not hFWE3, is briefly present at the plasma membrane where it assumes a four TM structure that positions both N- and C- terminal tails within the cytosol. Our results provide a framework for dissecting structure-based mechanisms of both hFWE-mediated cell competition and hFWE-dependent endocytic processes.

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Abstract 1440

Regulation of Proteostasis in the Cholesterol Biosynthetic Pathway

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UbiA prenyltransferase domain-containing protein-1 (UBIAD1) utilizes geranylgeranyl pyrophosphate (GGpp) to synthesize the vitamin K2 subtype menaquinone-4 (MK-4). Mutations in UBIAD1 cause Schnyder corneal dystrophy (SCD), which is characterized by corneal opacification owing to over-accumulation of cholesterol. Our studies disclosed a key role for UBIAD1 in regulating endoplasmic reticulum (ER)-localized, polytopic HMG CoA reductase, the rate-limiting enzyme in synthesis of cholesterol and nonsterol isoprenoids including GGpp. Feedback control of reductase involves sterol-induced ubiquitination, an obligatory reaction for its ER-associated degradation (ERAD) that is augmented by GGpp. Sterols also cause UBIAD1 to bind reductase, which inhibits ERAD and allows continued synthesis of nonsterol isoprenoids in sterol-replete cells. GGpp triggers release of reductase from UBIAD1, enhancing ERAD and stimulating translocation of UBIAD1 to Golgi. SCD-associated UBIAD1 resists GGpp-induced release from reductase and becomes sequestered in ER to inhibit ERAD. This inhibition results in enhanced synthesis and accumulation of cholesterol in cultured cells and tissues of knock-in mice expressing SCD-associated UBIAD1. The physiological significance of the reductase-UBIAD1 interaction is further highlighted by finding that ERAD-resistant reductase rescues embryonic lethality associated with Ubiad1 deficiency in mice. We recently reported the cryogenic electron microscopy structures of the reductase-UBIAD1 complex, which is maintained by interactions between transmembrane helices of UBIAD1 and reductase. Disrupting this interface prevents complex formation and enhances ERAD of reductase. The reductase contains a 170-amino acid sterol-sensing domain (SSD) that comprises transmembrane helices 2–6. The SSD of reductase exists in two conformations, one of which is essential for ERAD. These findings indicate rearrangement of transmembrane helices within the SSD allows recruitment of protein (s) that initiate reductase ERAD.

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Abstract 1455

Determining the binding interaction between *S. pombe* cationic amino acid transporter Cat1 and alpha arrestin Any1 by mutational analysis

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Morgan Morrison, Evan Guiney

The objective of this study was to determine how active transporters (pumps), located at the plasma membrane of a cell, are regulated. We use two species of yeast (*Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*) as models to study how such pumps are endocytosed. In eukaryotes, regulated endocytosis is commonly initiated once membrane proteins are ubiquitinated. This ubiquitination tag allows the cell to properly select membrane transporters and place them into endocytic vesicles. Ubiquitination is determined by a ubiquitin ligase/adaptor complex; featuring a core ubiquitin ligase enzyme bound to a variety of alpha-arrestin family adaptors which determine which pumps are targeted. Alpha-arrestin dependent ubiquitin mediated endocytosis of active transporters is best understood in *S. cerevisiae*; analysis of arrestin function in evolutionarily distant lineages such as *S. pombe* will provide insights into the conserved features of this common eukaryotic membrane trafficking system. Our previous studies in *S. cerevisiae* have suggested that a positively charged "basic groove" on the arrestin Art1 interacts with a negatively charged "acidic patch" binding site on the methionine pump Mup1. Charge inversion experiments provide key evidence for this model: adding positive charges to the Mup1 acidic patch completely blocks endocytosis, and compensating negative charges added to the Art1 basic groove restore it. The exact position of each charge inversion pairs is highly specific, suggesting "lock and key" binding. We set out to test whether this mechanism of binding also occurs in *S. pombe* for the pump/adaptor pair Cat1/Any1 (which are structurally similar and closely related to Mup1/Art1). Here we test the acidic patch/basic groove binding model by systematically mutating the cytoplasmic surface of the Cat1 pump. Course grain mutation mapping identified a 5 amino acid, negatively charged acidic patch that is indeed critical for endocytosis. This motif is distinct from the putative Cat1 ubiquitination site, and shares sequence features with the previously identified alpha arresting binding motifs on Mup1 and Can1 in *S. cerevisiae*. We are currently testing the effect of introducing positive charges in this region, and introducing corresponding negative charges into the hypothesized Any1 basic groove.

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Abstract 1483

Cannabidiol and LPS-Induced Inflammatory Responses in Cancerous Epithelial Cells

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CBD has been shown to impact cytokine responses in *in vivo* studies. In this study we examined the effects of two major CBD extracts (pure, and full spectrum) on cell viability and inflammatory cytokine response in a colon cancer cell line (T84) to elucidate whether the spectrum of CBD components influences these variables. Cannabis plants produce unique oils that contain terpenes, flavonoids, phenols, ketones, and other hydrophobic compounds. Pure CBD has no detectable terpenes or other compounds, broad spectrum CBD has no traces of THC but contains all the terpenes and flavonoids of a full spectrum CBD. Cell viability was evaluated at 4ug/ul and 6ug/ul of each formulation via flow cytometry, and non-cytotoxic doses of 4 and 6 ug/ul were used for all remaining experiments. LPS was used to induce cells to release cytokines following CBD treatment. Enzyme-linked immunosorbent assays were used to determine the concentration of Il-6, TNF-a and MCP-1produced by the epithelial cells following treatment under stimulated and non-stimulated conditions. Non LPS-stimulated cells treated with the different types of CBD show that TNF-a was decreased with the addition of pure CBD in the epithelial cells compared to controls not treated with CBD. MCP-1 and IL-6 concentrations were similar in CBD treated and untreated cells that were unstimulated. For the cells stimulated with LPS the full spectrum CBD lowered all cytokines when compared to the non-stimulated controls treated with both types of CBD compounds. TNF-a had the largest inhibition. The pure spectrum CBD lowered the cytokines but not as greatly as the full spectrum CBD. In LPS-stimulated cells, MCP-1 and IL-6 were similar to those seen in non-stimulated cells with both CBD compounds.

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Abstract 1484

Vph1NT is a novel PI(3,5)P2 biosensor that reversibly relocates in response to osmotic stress

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V-ATPases are highly regulated, ATP-driven proton pumps that acidify organelles. V-ATPases in the lysosome-like vacuole participate in the response to many stress signals, and V-ATPase activation in response to osmotic stress requires the vacuole-enriched signaling lipid PI(3,5)P2. The mechanism and dynamics of this stress response are poorly understood. The yeast vacuolar a-subunit isoform (Vph1) interacts with PI(3,5)P2 via its cytosolic N-terminal domain (Vph1NT), and this interaction stabilizes and activates the V-ATPase. In order to better understand the dynamics of the V-ATPase-PI(3,5)P2 interaction, we utilized a Vph1NT-GFP biosensor that interacts with lipid independent of other V-ATPase subunits. Under salt stress, PI(3,5)P2 levels transiently increase up to 20-fold. Vph1NT-GFP rapidly moves from the cytosol to membranes upon addition of salt. Using a microfluidic system, we observe that the extent and timing of Vph1NT-GFP recruitment is dependent on salt concentration. Importantly, recruitment is compromised in mutants defective in PI(3,5)P2 synthesis and in vph1 mutants that do not bind PI(3,5)P2. Interestingly, Vph1NT-GFP is consistently recruited to an area immediately adjacent to the vacuole. Recruitment is reversible, but upon washout and re-addition of salt, the biosensor returns to the same location. We propose that recruitment of Vph1NT-GFP to membranes reflects the stabilizing conformational changes that occur in response to Vph1 binding PI(3,5)P2 in the intact V-ATPase. These data suggest that osmotic stress may induce a short-term, localized activation of V-ATPase activity that could protect cells by driving vacuolar salt sequestration until transcriptional protection mechanisms can be activated. In addition, the Vph1NT-GFP biosensor can provide new insights into the localization and duration of PI(3,5)P2 signals *in vivo*.

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Abstract 1487

Role of calcium in mediating membrane tension and synaptic plasticity

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Neurons and other cells respond to cues from neurotransmitters and drugs through changes in calcium levels. Calcium responses can be mediated by the G protein/ phospholipase Cβ (PLCβ) signaling system, and this system is linked to schizophrenia and other neuronal disorders. To get insight into the role of calcium in mediating neuronal connections, we used a model neuronal cell line (PC12). We find that extended activation of the Gαq/PLCβ pathway disrupts synapses and causes neurites to retract into the cell body. Our studies show that this behavior results from increased membrane tension brought about by elevated calcium, PIP2 hydrolysis and receptor endocytosis. Several days after retraction the cells take on a stem-like morphology and produce stem cell markers. Changing membrane tension using osmotic stress instead of Gαq activation also causes retraction which we find depends on the smaller cytosolic volume and greater membrane contribution in the neurites versus the soma. We also find that many neurites display non-linear retraction in a pattern we refer to as 'squiggles'. Squiggling can also be explained through large discrepancies in the tension between the neurite and soma that set up tangential forces.

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Abstract 1493

Cyclin-dependent kinase 5 homolog Pho85 phosphorylates glycogen synthase kinase homolog Rim11

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Pho85 and Rim11 are the yeast homologs of mammalian cyclin-dependent kinase 5 and glycogen synthase kinase-3β, respectively. Pho85 is a multifunctional cyclin (e.g., Pho80)-dependent protein kinase involved in many signal transduction pathways affecting cell cycle progression and metabolism, whereas Rim11 is known to promote entry into meiosis by phosphorylating the transcriptional activator complex Ime1-Ume6. Pho85-Pho80 and Rim11 are also known to regulate lipid synthesis through the phosphorylation of Pah1, the yeast phosphatidate phosphatase that catalyzes the dephosphorylation of phosphatidate to generate diacylglycerol. The diacylglycerol produced in this reaction is used in the synthesis of triacylglycerol and the membrane phospholipids phosphatidylcholine and phosphatidylethanolamine. Mechanistically, the phosphorylations by Pho85-Pho80 and Rim11 control the location and phosphatidate phosphatase activity of Pah1. Preliminary studies indicate that phosphorylation of Rim11 by Pho85-Pho80 may regulate the Rim11-mediated phosphorylation of Pah1. In this work, we expressed and purified epitope-tagged versions of both protein kinases from *Escherichia coli* to examine in the phosphorylation of Rim11 by Pho85-Pho80. The Pho85-Pho80-mediated phosphorylation of Rim11 was time- and dose-dependent and dependent on the concentrations of Rim11 and ATP. Thus, Rim11 is a bona-fide substrate of Pho85-Pho80. The sites of phosphorylation on Rim11 are being determined for the purpose of constructing site-specific mutations to study the physiological consequences of phosphorylation by Pho85-Pho80 and the phosphorylation of Pah1.

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Abstract 1495

The Role of KCNE4's Tetraleucine Motif in Dimerization

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Accessory subunits belonging to the KCNE family modulate voltage-gated potassium (Kv) channels such as Kv1.1, Kv1.3, Kv2.1, and Kv7.1. Incorrect modulation of potassium current through these channels have been linked to several diseases for example, long QT syndrome, allergic rhinitis, and acute lymphoblastic leukemia. Increased understanding of the molecular underpinnings of KCNE modulation of potassium channels would help expand our understanding of the etiology of these diseases. KCNE4 is unique in the KCNE family as it has been shown to inhibit current in Kv channels. Previously, the role of KCNE4's tetraleucine motif has been tied to its inhibitory effects and interaction with Kv channels. Roig et al., 2021, suggests that this tetraleucine motif drives homodimer formation of KCNE4 before association with calmodulin or Kv channels. In this study, we elaborate on this hypothesis by measuring distance distributions between KCNE4 mutants with (L4+) and without (L4-) the tetraleucine motif using double electron-electron resonance (DEER) spectroscopy. DEER spectroscopy is a pulsed electron paramagnetic resonance (EPR) technique used to measure the distance between two or more paramagnetic centers. This method in combination with SDS-PAGE and line-shape analysis of continuous-wave EPR (CW-EPR) spectroscopy provides site specific information of the structure, dynamics, and conformation of dimerization. Our current data suggests that the tetraleucine motif may drive dimer formation and/or stability but is not the only factor. The distances, oligomer conformation, and dynamics of KCNE4 mutants with and without the tetraleucine motif determined in this study will be used to elucidate modulation of Kv channels in native and disease states.

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Abstract 1499

Resolving Phospholipase Cbeta Regulation by G Proteins and the Membrane

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Phospholipase C β (PLCβ) increases intracellular calcium in response to diverse hormones, regulating numerous processes including cell proliferation and survival. Dysregulation of its expression or activity contributes to heart disease, cancer, and other pathophysiological conditions. PLCβ interacts with the cytoplasmic leaflet of the plasma membrane, where it hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2) to produce second messengers that increase intracellular Ca^{2+} and activate protein kinase C. PLCβ exists in an autoinhibited state that is relieved via direct interactions with the heterotrimeric G protein subunits, $G\alpha_q$ and Gβα, and the membrane, coupling lipase activity to stimulation of G protein-coupled receptors. Prior structural and functional studies have revealed how Gαq binds to and activates PLCβ, but where Gβg binds to PLCβ to regulate its activity is controversial. Using cryo-electron microscopy, we have determined a series of reconstructions of the Gβg–PLCβ complex, allowing identification of the Gβg binding surface, as well as an unexpected role for Gβg as a potential scaffold for PLCβ, wherein a pre-activated complex is maintained at the membrane. We are also using single molecule approaches to investigate the behavior of PLCβ on model membranes, as maximum activation only occurs when the lipase interacts with both the membrane and a G protein activator. Taken together, these studies provide the first mechanistic insights into the coordinated regulation of PLCβ by G proteins and the membrane.

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Abstract 1502

Misrouting of Niemann-Pick C1 protein mediates cholesterol induced mTORC1-activation contributing to pathogenesis of CLN1-disease

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The CLN1-disease is a neurodegenerative lysosomal storage disorder (LSD). it is caused by inactivating mutations in the CLN1 gene encoding palmitoyl-protein thioesterase-1 (PPT1), a lysosomal enzyme that depalmitoylates S-palmitoylated proteins. Despite this discovery, the pathogenic mechanism of CLN1-disease has remained elusive. Recently, it has been reported that cholesterol mediates mTORC1-activation, which suppresses autophagy. Suppressed autophagy mediates neurodegeneration in most LSDs. We found that total cholesterol in the brain of Cln1-/-mice, which mimic CLN1-disease, is significantly elevated. Intriguingly, in Cln1-/- mice, the level of NPC1 in lysosomal fractions was significantly lower compared with that in their WT littermates. Lysosomal cholesterol homeostasis is maintained by Niemann Pick C1 (NPC1)- and NPC2-proteins, which mediate cholesterol egress and import, respectively. We found that NPC1 requires dynamic S-palmitoylation for trafficking to the lysosomal surface. We also found that in Cln1-/- mice, lack of Ppt1 misrouted NPC1-protein to the plasma membrane instead of its normal location on lysosomal membrane. However, the level of NPC2, a lysosomal cholesterol importer, was elevated in Cln1-/- mice, disrupted lysosomal cholesterol homeostasis. Since endosomal trafficking of proteins to the lysosomal membrane requires sequential interaction with various adaptor proteins (APs), we investigated AP-mediated trafficking of NPC1 in WT and Cln1-/- mice. We found that Ppt1-deficiency in Cln1-/- mice lack of dynamic S-palmitoylation of NPC1 prevented its handover from AP-2 to AP-3. Consequently, NPC1 failed to be transported to lysosomal membrane and misrouted to the plasma membrane. As a result, the lack of NPC1 on the lysosomal membrane elevated the level of oxysterol binding protein (OSBP) and its anchoring proteins-VAPA and VAPB, which increased cholesterol level on lysosomal surface, mediating mTORC1-activation. The activation of mTORC1 suppressed autophagy contributing to neurodegeneration. To determine whether inhibition of OSBP may rescue autophagy, we treated cultured INCL lymphoblasts and Cln1-/- micewith OSW1 (pharmacological inhibitor of OSBP) and evaluated the levels of mTORC1-signaling and autophagy. The results showed a significant decrease of mTORC1-activation markers, pS6K1 and p4E-BP1 and autophagy markers, LC3B and p62 in the OSW1 treated lymphoblasts from patients with CLN1-disease and in Cln1-/-mice compared with normal lymphoblasts and WT mice, respectively. Importantly, we found that pharmacological inhibition of OSBP suppressed mTORC1 activation and rescued autophagy protected the neurons in the brain of Cln1-/- mice. Our findings uncover a previously unrecognized role of Cln1/Ppt1 in lysosomal cholesterol homeostasis and reveal a pathway to pathogenesis of CLN1-disease.

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Abstract 1504

Septin filaments interact with AP-3 and are required for trafficking via the AP-3 pathway in budding yeast

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AP-3 is an adaptor protein complex that is structurally and functionally homologous to the AP-1 and AP-2 complexes, which sort cargoes into clathrin-coated vesicles and often fuse with endosomes. However, AP-3 functions independently of clathrin and sorts cargoes that fuse with the lysosome and lysosome-related organelles. Like AP-1 and AP-2, AP-3 is predicted to function with numerous accessory proteins that facilitate vesicular transport, but few of these accessory proteins have been identified. The objective of this project was to identify new accessory proteins that drive AP-3 cargo sorting. To accomplish this objective, we used mass spectrometry to identify proteins that bind AP-3 subunits from lysates of *Saccharomyces cerevisiae*. Among the proteins co-purified were all five septin proteins expressed in mitotically growing yeast. Septins were discovered and named for their function at the plasma membrane during cytokinesis. Experiments based on bimolecular fluorescence complementation (BiFC) confirmed septins interact with AP-3 *in vivo* and showed their interaction occurs at discrete puncta frequently located adjacent to vacuoles, which is distinct from septin interactions observed at the plasma membrane. Septin genes with point mutant alleles were also identified in a genetic screen looking for mutants that produce an AP-3 sorting defect, confirming a functional role for septins in the AP-3 pathway. Thus, our studies have identified septins as accessory proteins that function with AP-3.

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Abstract 1516

Did Phospholipids Evolve to Increase the Stability of Fatty Acid Membranes?

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E. Eugene Williams

Currently on Earth, all cells are encapsulated in a phospholipid bilayer. Phospholipids possess amphipathic characteristics that allow them to interact with both aqueous and nonaqueous environments. In prebiotic earth phospholipids were not available for membrane construction because they are only produced by biotic mechanisms. Thus, protocells (prebiotic precursors of cells) could not have had membranes that were composed of phospholipids. Instead, it is believed that single chain amphiphiles, such as fatty acids, were the building blocks for primitive cellular membranes. It is known that fatty acids have the ability to encapsulate cells and can readily form bilayer membranes. Fatty acid membranes are stable in water but are unstable and form life-incompatible micelles in aqueous solutions above pH 10 and in solutions containing even millimolar concentrations of Ca+2 or Mg+2. It is possible that increasing proportions of phospholipid stabilize the membrane against these otherwise adverse conditions. To test this hypothesis, fatty acid vesicles (stearic acid) with increasing proportions of phospholipids (distearoyl phosphatidylcholine) were prepared and suspended in 0.1 M TRIS buffer at pH 8.5. These vesicles were then titrated to pH 12.0 with NaOH. Membrane stability, the transition from vesicles to micelles, was monitored with two independent methods: turbidity at 400 nm and Nile red fluorescence. The results show that the addition of phospholipids aided in the stabilization of the vesicle membranes. These results help provide an understanding of the possible composition and evolution of protocell membranes.

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Abstract 1556

Phosphatidate phosphatase Pah1 contains an inhibitory domain that regulates its function in yeast lipid synthesis

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The *Saccharomyces cerevisiae* phosphatidate (PA) phosphatase enzyme, Pah1, catalyzes the dephosphorylation of PA to produce diacylglycerol, which is the precursor for the energy storage molecule triacylglycerol as well as the membrane phospholipids phosphatidylcholine and phosphatidylethanolamine. Pah1 function is predominantly regulated by its subcellular localization through the posttranslational modification of phosphorylation and dephosphorylation. Phosphorylated Pah1 is stable in the cytosol and recruited to and dephosphorylated by the Nem1-Spo7 phosphatase complex in the nuclear/ER membrane. The dephosphorylated Pah1 associates with the membrane, recognizes the substrate PA, and catalyzes its dephosphorylation. During transition from the exponential to the stationary phase of cell growth, Pah1 translocation is increased for increased function. Using bioinformatics, we identified a novel domain (named I-domain) of Pah1 within the intrinsically disordered region between the two conserved domains. In functional analyses, the expression of Pah1 deficient in I-domain (Pah1-IΔ) rescued the pah1Δ mutant but did not require the Nem1-Spo7 activity. Mass spectrometry analysis showed that the phosphorylation state of Pah1-IΔ was significantly different from the WT enzyme. We examined the abundance and localization of Pah1-IΔ to determine the effect of the I-domain on Pah1 stability and its translocation, and constructed substitution mutations in the I-domain to identify key residues in the regulation of Pah1 phosphorylation, translocation, and function in lipid synthesis.

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Abstract 1613

Regulation of lysosomal lipids by the α1,6-Mannosyltransferase Alg12

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Jessica Davidson, Judith Simcox

Lysosomes are essential organelles in the cell that perform tissue-specific roles in macromolecular degradation, transport, and cellular signaling. An important component of lysosomes are bis(monoacylglycero)phosphates (BMP) lipids. BMPs are anionic glycerophospholipids that are found in intraluminal vesicles of late endosomes and lysosomes, and are involved in sphingolipid degradation, cholesterol transport, and lysosomal membrane dynamics. BMP lipid levels are increased in lysosomal storage disorders, nonalcoholic fatty liver disease, and cardiovascular disease. Physiological stresses such as cold exposure, high-fat diet, and fasting can also increase BMP levels. Despite evidence of their crucial role in lysosomal function, key enzymes and proteins involved in the synthesis and regulation of BMP lipids are unknown. We performed quantitative trait loci analysis of high fat diet fed mice using genomics and lipidomic datasets to map several genetic regulators of hepatic BMPs. We identified the α1,6-mannosyltransferase Alg12, an N-linked glycosylation enzyme, as a candidate. Using targeted liquid chromatography-mass spectrometry (LC-MS) to quantify BMP lipids, we found that overexpressing Alg12 led to increased BMP levels while the lysosomal abundance remains unchanged. Alg12 is involved in trafficking proteins to the lysosome through the mannose 6-phosphate receptor pathway. We hypothesize that Alg12 is modulating BMP lipid levels through targeting a regulator to the lysosome. To identify the Alg12 target responsible for altered BMP lipid levels, we will assess glycoproteomics of Alg12 overexpression and null cells. Understanding BMP regulation by Alg12 will bring new insights into the contribution of BMP lipids to lysosomal function.

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Abstract 1634

Effect of lipid raft disrupting agents on FSHR-dependent cAMP production

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Infertility affects millions of individuals worldwide and the causes of infertility are often unexplained. There are a number of signaling pathways that are involved in reproduction and defects in these pathways could contribute to infertility. Specifically, we are interested in studying how FSHR (follicle stimulating hormone receptor) responds to human follicle stimulating hormone (hFSH) because having the ability to enhance or downregulate its signaling has implications for fertility treatments. When FSH binds to its cognate G-protein-coupled receptor (GPCR) it activates the production of cAMP as a second messenger. Previous work in our lab has demonstrated that FSHR needs to be embedded in a specialized microdomain of the cellular membrane called a lipid raft to mediate this response. After treating FSHR-expressing cells with lipid raft-disrupting drugs, decreased cAMP was observed as a decrease in activated (phosphorylated) cAMP response-element binding protein (pCREB) within the Gα/cAMP/PKA pathway. The amount of pCREB is an indirect measurement of cAMP production. To establish a direct relationship between FSHR signaling and cAMP production, a quantitative luciferase assay was developed. Luciferase is an enzyme found in fireflies that allows them and other bioluminescent organisms to glow. A cell line expressing hFSHR (HEK293-hFSHR) was transiently transfected with a plasmid to express luciferase under the control of multiple, tandem cAMP response elements. This acts as a quantitative reporter for cAMP because it produces more luminescence as the amount of cAMP in the cells increases. Cells were treated with Methyl-β-cyclodextrin (MβCD) which acts as a lipid raft-disrupting agent by depleting cholesterol from cells and destroying lipid rafts. All cells were stimulated with various amounts of FSH to create a dose-response curve. MβCD-treated cells produced less cAMP in a dose-dependent manner compared to the untreated cells. These results differ from previous observations of the FSH-induced activation of p44/42 MAP kinase (ERK1/2). It was found that treatment with the lipid raft disrupting agents resulted in increased basal cAMP production (as measured by pCREB activation). Future studies will test different lipid raft-disrupting agents to further investigate the role of lipid rafts in FSH induced cAMP production from FSHR stimulation. Studying and characterizing FSHR behavior is essential to furthering our understanding of infertility and improving the possible treatments for both men and women who are struggling to conceive.

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Abstract 1653

The phosphorylation of yeast Pah1 phosphatidate phosphatase by Pho85-Pho80 is attenuated by a synthetic peptide containing the protein kinase target sites

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Gil-Soo Han, George Carman

The yeast phosphatidic acid (PA) phosphatase, Pah1, regulates the synthesis of triacylglycerol and phospholipids by controlling the relative amounts of its substrate PA and product diacylglycerol (DAG). Pah1 phosphorylation is mediated by multiple protein kinases, and its phosphorylation status regulates its localization, catalytic activity, and stability. Prior work has shown that the Pho85-Pho80 protein kinase complex phosphorylates Pah1 at seven sites (Ser-110, Ser-114, Ser-168, Ser-602, Thr-723, Ser-744, and Ser-748) to stabilize and localize it to the cytoplasm where it is not functional. Phosphorylated Pah1 is recruited to the nuclear/ER membrane through its association with the Nem1-Spo7 protein phosphatase complex; the complex dephosphorylates Pah1 at the seven sites. Following the dephosphorylation, Pah1 hops onto to membrane for association with and dephosphorylation of its substrate PA to produce DAG. In this study, we examine the hypothesis that a peptide containing the seven target sites of phosphorylation by Pho85-Pho80 could compete with Pah1 for its phosphorylation by the protein kinase. A synthetic gene encoding a 63 amino acid-long peptide that contains the seven phosphorylation sites and a GFP sequence at the C-terminus was expressed in yeast cells. Confirmation of the peptide expression was scored by intracellular fluorescent labelling. Phenotypic analyses of the peptide effects include the phosphorylation status and location of Pah1 and the relative synthesis of triacylglycerol and membrane phospholipids.

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Abstract 1706

Characterization of Select Lysine Mutations of the Cystine/Glutamate Transporter, System xc-

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System xc- is a membrane transport system that plays a critical role in mitigating oxidative stress and controlling the level of glutamate, an excitatory neurotransmitter, in the brain. As such, its regulation is critical for proper brain functioning. Recent work in our lab has shown that System xc- activity increases immediately during an oxidative insult, and this process is necessary to maintain appropriate levels of antioxidants within neurons and glial cells. We have also shown that during the oxidative insult, System xc- undergoes a change in localization to the plasma membrane which allows for an increase in transporter activity, but we have yet to identify the specific mechanism for the redistribution of the transporter. Previous studies have demonstrated that post-translational modifications of proteins can lead to differential protein distribution within cells. Therefore, in this study, we sought to determine if post-translational modification (PTM) of the transporter regulates its trafficking. First, we identified four conserved lysines (K37, K422, K472, K473) which exhibit decreased activity upon mutation to arginine, suggesting that PTM of these sites increases activity. Next, we expressed the constructs in cultured mammalian cells and used biotinylation to examine the effects of the mutations on transporter localization in the cells. We also evaluated the effects these mutations had on the tendency for these transporters to undergo PTM. Collectively, this approach allows us to directly relate changes in PTM status at the select lysines with changes in transporter localization so that we can better understand the specific mechanism by which oxidants regulate System xc-. We demonstrated that these mutants exhibited no changes in their ubiquitination status, suggesting that another PTM must be impacted. However, we observed that K473R exhibits a 5–10 kD decrease in the molecular weight, indicating that K473 is modified under basal conditions. K472R and K473R do not appear to shift to the membrane following peroxide treatment and may be stuck in the endoplasmic reticulum. Our preliminary results indicate that K37R exhibits reduced membrane localization relative to wildtype but still appears to shift to the membrane following peroxide exposure. Further analysis of K422R will be done to assess transporter localization. We are currently working to identify the specific PTM that occurs on K473, such as Neddylation or SUMOylation, and the impact this PTM has on transporter localization. In summary, PTM of K37, K472, and K473 seem to support xCT delivery to the membrane under basal conditions, and we have yet to identify how K422 impacts transporter trafficking.

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Abstract 1720

Identification of the stearoyl-CoA desaturase in *Schizosaccharomyces pombe*

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The fatty acid desaturase catalyzes the rate limiting step in the production of unsaturated fatty acids. We screened for potential fatty acid desaturase genes in *Schizosaccharomyces pombe* by looking for homology to a known fatty acid desaturase from the distantly related yeast *Saccharomyces cerevisiae*. Two candidate genes were identified: SPBC3B8.07c (also identified as dsd1, encoding the dihydroceramide delta-4 desaturase) and SPCC1281.06c. Mutants in both candidate genes were screened for unsaturated fatty acid auxotrophy; the dsd1 mutant did not exhibit any growth defects when grown in the absence of UFAs, but the SPCC1281.06c mutant did. In addition, the two candidate genes were isolated and screened for the ability to complement an ole1Δ desaturase deficient strain of *Sacc. cerevisiae*. Only the SPCC1281.06c was capable of complementing the ole1Δ mutant in *Sacc. cerevisiae*. Initial characterization of the regulation of expression of SPCC1281.06c by exogenous unsaturated fatty acids is presented. We conclude that the SPCC1281.06c gene encodes a stearoyl-CoA desaturase, and is the sole stearoyl-CoA desaturase in *S. pombe*.

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Abstract 1758

Development of a mitosis-specific plasma membrane recruiter

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Mitosis is a series of highly ordered events regulated by myriad pathways, including phospholipid signaling at the plasma membrane (PM). Several phospholipids, including phosphatidylethanolamine and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2), are important for successful cytokinesis. However, our understanding of the functions of these lipids during earlier stages of mitosis is limited, partly due to a lack of tools to manipulate phospholipid composition at the PM specifically during mitosis. Recently, we found that a PI(4,5)P2-binding protein, pleckstrin homology domain-containing family A, member 5 (PLEKHA5), exhibited cell cycle-dependent, conditional PM localization, with the protein exclusively localized to the PM during mitosis and minimally during interphase, when the protein is localized to the microtubule cytoskeleton. Here, we investigated the molecular mechanism regulating the dynamic localization of PLEKHA5 and harnessed this unusual behavior to develop a reversible, mitosis-specific PM recruiter based on PLEKHA5 domains capable of modulating cargo protein recruitment to the PM during mitosis. Mechanistically, we found that membrane association of PLEKHA5 was dependent upon the phosphorylation status of a single serine residue located adjacent to the PLEKHA5 pleckstrin homology domain. Further, modulation of protein kinase C and casein kinase 1 activity suggests roles for both of these kinases in this phosphorylation. These mechanistic studies identify PLEKHA5 as a protein wherein a functional phosphorylation event decreases during mitosis, running counter to the prevailing understanding of mitosis as a setting for, in general, increased protein phosphorylation. Next, to harness the dynamic PM-binding capability of PLEKHA5, we engineered a mitosis-specific PM recruiter comprising the minimal PM-binding domains of PLEKHA5 with additional point mutations and a nuclear export signal. This engineered PM recruiter exhibited reversible shuttling between the cytosol during interphase and the PM during mitosis. Fusion of a GFP nanobody to the PM recruiter enabled successful PM recruitment of GFP-tagged cytosolic proteins during mitosis. These studies point to future applications of this tool for mitosis-specific PM recruitment or "knock-sideways" of desired target proteins, including potentially phospholipid-modifying enzymes or other effectors capable of modulating cell signaling pathways.

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Abstract 1790

Human Follitropin Receptor Lipid Raft Residency is Mediated by its Caveolin Binding Motif

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Brian Cohen

The follicle stimulating hormone (FSH) is a necessary hormone involved in reproductive pathways in both males and females. Previous work in our lab has shown that its receptor (FSHR) contains a caveolin binding motif (CBM) which we believe is responsible for localization of the receptor to lipid raft domains of the membrane and for proper receptor signaling. Mutations were introduced to this motif to provide a deeper understanding of this interaction and identify the importance of specific sites within the CBM in FSHR. Discontinuous sucrose gradients were used to separate lipid raft from non-lipid raft portions of the membrane and western blotting was used to visualize the localization of the FSHR within the gradient. We found that disruption of the CBM of FSHR resulted in altered localization of the receptor relative to the wild type FSHR. Receptors with mutations F497L, F481L, and F486L, as well as F497L, F481L, F486L, and F489L were found to localize only to non-raft portions of the membrane, while receptors with mutations F481L, F486L, and F489L were found in both lipid raft and non-raft portions of the membrane. From this we concluded that F479 may play a more significant role in lipid raft localization and signaling. These findings are consistent with previous research within the lab, which show higher levels of basal signaling in CBM mutants compared to the wild type. The results of this study suggest that the CBM is important in proper signaling of FSH, and that certain sites within the CBM may be more important in this interaction than others. These findings are important in understanding FSHR function which could lead to the development of novel treatments for infertility.

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Abstract 1809

Lomitapide and high-density lipoprotein function in homozygous familial hypercholesterolaemia

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Annalisa Ronca, Matteo Incerti, Alessandra Rossi, Matteo Manfredini, Elda Favari

Background Lomitapide is an inhibitor of microsomal triglyceride transfer protein reduces plasma low-density lipoprotein cholesterol (LDL-C) and is approved for the treatment of homozygous familial hypercholesterolemia (HoFH). Lomitapide can affect high-density lipoprotein (HDL) composition and function in HoFH. The present study aims to determine the effect of lomitapide on HDL and cholesterol efflux in a cohort of patients with HoFH. Patients and methods Analysis included plasma samples from 17 HoFH patients enrolled in the lomitapide phase 3 study (NCT00730236). At baseline, 17 HoFH patients were receiving LDL apheresis. Samples taken at baseline (pre-lomitapide) and Weeks 56 and 66 (assumed steady-state on lomitapide) were analysed for HDL levels and cholesterol efflux capacity (CEC) via ATP-binding cassette transporter A1 (ABCA1), ABCG1, scavenger receptor class B type I (SR-BI) and aqueous diffusion from various cell lines. Results In patients not undergoing apheresis (n = 7), mean levels of LDL-C decreased in response to lomitapide (LDL-C: 211.7 mg/dL [low dose] and 149.8 mg/dL [high dose] versus 327.7 mg/dL at baseline; and HDL-C moderately decreased or remained close to baseline: 40.1 mg/dL and 44.3 mg/dL versus 45.3 mg/dL at baseline). In patients undergoing apheresis (n = 11), levels of LDL-C decreased mostly at higher doses of lomitapide 40–60 mg (baseline 319.7 mg/dL versus 165.7 mg/dL Weeks 56–66). This was associated with a decrease in apoB containing particles in patients not receiving apheresis vs receiving apheresis (295.6 g/L) vs (242.7 g/L) respectively. Lomitapide decreased ABCA1-mediated CEC in all patient's plasma and promotes the SR-BI pathway (Wald $\chi 2$ $p < 0.01$). However, ABCG1- and total efflux did not change consistently. Conclusion Lomitapide reduced LDL-C and apoB levels independently from apheresis procedure. Our report raises the hypothesis that lomitapide promotes a shift in HDL to larger HDL particles able to interact with SR-BI but not with ABCA1. This shift does not impair total efflux process indicating that lomitapide drives the last part of the RCT through SR-BI receptor in HoFH patients. This suggested that HDL lipids with lomitapide are mostly removed from plasma through the catabolism of apoB containing particles which may influence the capacity of plasma to affect CEC from macrophages.

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Abstract 1866

Loss of CLN1/PPT1 dysregulates anterograde protein-trafficking causing ER-stress and UPR in CLN1 disease

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Abhilash Puthuvelvippel Appu, Maria Bagh, Avisek Mondal, Anil Mukherjee

Neuronal Ceroid Lipofuscinoses (NCLs) represent a group of the most common neurodegenerative lysosomal storage disorders. Mutations in at least 13 different genes (called CLNs) cause various types of NCLs. Mutations in the CLN1 gene encoding a lysosomal depalmitoylating enzyme, PPT1, cause CLN1-disease. Previously, we reported that ER-stress mediates unfolded protein response (UPR) in the brain of Cln1-/- mice, which mimic CLN1-disease. In the present study, we tested a hypothesis that in CLN1-disease either defective ER-Golgi or Golgi-ER trafficking mediates ER-stress. The newly synthesized proteins from the ER are transported to the Golgi via COP II vesicles. Subsequently, they are transported from the Golgi to the endosomal compartments. Retrograde transport (Golgi to ER) may also occur via COP I vesicles. Accumulation of unfolded proteins in the ER may induce ER-stress. Fibroblasts from patients with CLN1 disease suffer from ER-stress causing unfolded protein response (UPR) promoting neuronal apoptosis. We found that COPII-mediated ER to Golgi transport of proteins was impaired in cultured fibroblasts from patients with CLN1-disease. We found that the sequential recruitment of COPII complex-proteins (e. g. Sar1, Sec23/Sec24 subcomplex, and Sec13/Sec31 subcomplexes) were significantly increased in Cln1-/- mouse brain compared with those of WT mice. Colocalization studies using CLN1-disease fibroblast showed that all COPII-associated proteins were colocalized at a higher level with the ER-marker in CLN1-disease fibroblasts compared with that of normal fibroblasts. To further confirm these results, we isolated the ER-fraction and measured the level of Sar1, Sec31A, Sec 24A and Sec 23A. We found that the levels were significantly elevated in the ER-fractions, except for Sec13, which was not elevated in the ER. We previously reported that Ppt1-deficiency misrouted V0a1 subunit of v-ATPase to the plasma membrane (PM) instead of its normal location on lysosomal membrane (Bagh et al. Nat Commun, 2017). We analyzed whether the COPII components are S-palmitoylated and the results showed that Sar1, Sec31A, Sec23A and Sec24A are S-palmitoylated proteins. Intriguingly, like V0a1, the COPII

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components were also mis-localized to the PM. Further, the S-palmitoylated COPII components from CLN1-patient fibroblasts as well as those from Cln1-/- mouse brain was colocalized with the PM-marker. Our results confirm and extend the previous findings suggesting that Ppt1-deficiency contributes to misrouting of S-palmitoylated proteins to the PM instead of its normal destination. Consistent with these results, the UPR elements were also markedly elevated in Cln1-/- mouse brain. We conclude that defective ER-Golgi trafficking causing abnormal accumulation of misfolded proteins in the ER contributes to ER-stress, which activates UPR contributing to pathogenesis underlying CLN1-diseases.

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Abstract 1891

Hunting for genes encoding microbial membrane lipid pathways for synthetic biology applications

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Bacterial membranes can present a large diversity of amphiphilic lipids, including the common phospholipids phosphatidylglycerol, phosphatidylethanolamine and cardiolipin, the less frequent phospholipids phosphatidylcholine, and phosphatidylinositol and a variety of other membrane lipids, such as for example glycine lipids, ornithine lipids, glycolipids, sulphonolipids, sphingolipids or hopanoids among others. The presence or absence of these lipids can influence membrane properties such as fluidity or permeability or the interaction of the bacteria with eukaryotic hosts. For more than a decade we have been searching for, identifying and characterizing genes and enzymes involved in membrane lipid synthesis and modification from a wide diversity of bacteria such *Rhizobium tropici*, *Streptomyces coelicolor*, *Serratia proteamaculans*, *Vibrio cholerae*, *Singulisphaera acidiphila*, *Burkholderia cenopacia and Flavobacterium johnsoniae*. Here we present some of our most recent findings with respect to glycine and ornithine lipid and sulphonolipid synthesis and function and the proposal to use these genes in a synthetic biology approach.

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Abstract 1953

Cell penetration and nucleus delivery of DNA methyltransferases for *in vivo* chromatin mapping

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Recent advances in single-molecule chromatin fiber sequencing have enabled the study of gene regulation along multi-kilobase chromatin fibers at near single-nucleotide resolution. These technologies rely on the use of sequence-specific or non-specific methyltransferases (MTase) to label bases along chromatin fibers that are unbound by nucleosomes, transcription factors, or other DNA-binding proteins. Although these technologies provide valuable information on chromatin structure, gene regulation is dynamic, and appropriately studying these dynamic processes requires a technique that does not necessitate nuclei isolation prior to enzyme treatment. To overcome this, we have engineered MTases that can undergo cell penetration and nucleus delivery upon incubation with living cells, thereby enabling the labeling of chromatin fiber architectures and dynamics within living cells over a matter of minutes. Specifically, we have tested different strategies for *in vivo* delivery of engineered MTases, including MTase supercharging, as well as MTase fusions to diverse cell-penetrating peptides (CPP) and nuclear localization signal peptides. We find marked variability in the penetration efficiency between these different approaches, and find that treatment of human cells with an optimized CPP-tagged MTase results in methylation of only 10–20% of chromatin fibers, suggesting that although this protein is likely efficiently taken up in the endosomes, it is not being consistently trafficked to the nucleus in most cells. Single-molecule chromatin fiber-sequencing of cells treated with CPP-MTases for various time lengths enables us to resolve nucleosome dynamics genome-wide, disentangling the contribution of nucleosome site exposure and eviction at promoter-proximal gene regulatory elements. Overall, our approach provides a framework for studying chromatin architectures within living cells at single-molecule and single-nucleotide resolution and highlights challenges associated with the efficient delivery of functional MTases as cargo.

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Abstract 2001

A Conformational Disease Component of Lowe Syndrome Pathology Rescued by Allosteric Activator Candidates

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Lowe syndrome (LS) is a lethal disorder with no cure. Afflicted patients exhibit multisystem issues which primarily affect the eyes, brain, and kidneys. LS is caused by mutations in the OCRL1 gene, found on the X-chromosome, which encodes for an inositol polyphosphate 5-phosphatase of the same name. Among the various disease-causing alterations that have been identified, numerous missense mutations have been found in the 5-phosphatase catalytic domain, of which only half are in residues essential for lipid binding and catalysis. For these mutations, we hypothesize that there is an altered conformational equilibrium in LS pathogenesis, whereby a conformer incapable of proper biological function is predominant. Therefore, we aimed to characterize mutational consequences of patient Ocrl1 variants. First, to determine whether catalytic activity was affected, we purified recombinant, bacterially produced wildtype (WT, Ocrl1WT) and mutant (Ocrl1MUT) protein for *in vitro* testing. Ocrl1MUT fell into three major subgroups, those exhibiting no activity (I393F, D451G, V508D, Y513C, I533T), partial activity (M299I, D431N), and WT levels of activity (S256N). From here, we transfected OCRL1-null HK2 cells to evaluate the cellular effects of these mutations. Golgi apparatus (GA) fragmentation and primary cilia defects have previously been identified for OCRL1-null cells, so these phenotypes were assessed. GA fragmentation was consistent with biochemical results where inactive variants demonstrated more severe abnormalities compared to those with partial or complete activity. On the other hand, primary cilia defects were seen across Ocrl1MUT. Next, considering our hypothesis, we speculated that allosteric activators capable of shifting the conformational equilibrium towards the active conformer could rescue the mutational consequences of a number of these Ocrl1 patient variants. Several large-scale, biochemical drug screens were pursued for the identification of potential activators of Ocrl1D451G, from which we have selected four primary candidates. These drug candidates, in particular candidate LC9, could rescue the catalytic activity and GA fragmentation of all tested Ocrl1MUT. Additionally, the chemical chaperone 4-phenylbutyric acid could do the same, albeit to a lesser degree than candidate drugs. Altogether, these drug tests support a conformational component in the LS disease model that is recoverable by allosteric activation. Furthermore, biochemical tests were carried out in the presence of the isolated phosphatase domain, suggesting that drug rescue is a result of direct interaction with the catalytic domain. Overall, our study demonstrates the heterogeneity of protein dysfunction based on

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specific mutations and presents a new conformational component of LS pathogenesis. Candidate drugs identified and evaluated here offer novel therapeutics for LS whereby the drug directly targets Ocrl1 rather than downstream effects of OCRL1 mutations.

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Abstract 2020

Mechanistic insights into BECN2:Syntaxin6 complex-mediated vesicle fusion during non-canonical autophagy

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Syntaxin 6 (STX6), a SNARE protein, interacts with BECN2, a protein that functions in canonical and non-canonical autophagy. This interaction has been shown to function in non-canonical autophagy through the ATG9A-bearing vesicles. Here, we show that BECN2 interacts with STX6 via the BECN2 coiled-coil domain (CCD) and the STX6 SNARE domain. The STX6 SNARE is disordered in that absence of interacting partners, but becomes helical when in complex with the BECN2 CCD. Based on their melting temperatures, the BECN2 181-250:STX6 SNARE complex is more stable than either the BECN2 CCD homodimer, the BECN2 181-250 homodimer, or the un-complexed STX6 SNARE. Further, we present a 2.65 Å X-ray crystal structure of the BECN2 181-250:STX6 SNARE complex showing it forms a heterotrimeric helical bundle, comprised of one BECN2 181-250 and two STX6 SNAREs that are anti-parallel to each other. The heterotrimer interface is stabilized by 15 layers of three residues, each of which is contributed by a different helix. Of these 15 layers, six are comprised entirely of hydrophobic residues, including two layers that consist of three leucine residues each. We verified the role of these hydrophobic layers using point mutations and affinity pulldowns, showing that mutations of the hydrophobic layers either significantly or completely disrupt the interaction between BECN2 181-250 and STX6 SNARE. It is likely that this three-helix bundle represents a stable intermediate during the formation of the full SNARE complex, thereby providing mechanistic insights into the process of non-canonical autophagy involving the ATG9A-bearing vesicles.

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Abstract 2047

Cryo-EM structure of an endogenous mycobacterial MCE lipid transporter

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, is one of the leading causes of death due to infectious diseases. The Mammalian Cell Entry (MCE) proteins are essential virulence factors during replication within host macrophages and are important for the import of nutrients such as fatty acids and cholesterol across the mycobacterial cell envelope, which is largely impermeable. The mce1 – mce4 gene clusters are among the most complicated operons in the Mtb genome, encoding 9 to 13 proteins. Additional subunits are encoded throughout the genome, and it is unclear how all these proteins assemble into a complex that can move hydrophobic molecules across the cell envelope. Using *Mycobacterium smegmatis* as a model for Mtb, we tagged MCE subunits in the bacterial chromosome, purified an endogenous MCE complex using these tags, identified components of the Mce1 protein complex, and determined its structure using single-particle cryo-electron microscopy, mass spectrometry, and AlphaFold. The ~2.7 Å structure reveals how the proteins of the Mce1 fatty acid import system assemble to form an elongated, transenvelope ABC transporter complex. The Mce1 complex is dominated by a curved, needle-like domain that appears to be unrelated to previously described protein structures and creates a protected hydrophobic pathway for lipid transport across the periplasm. Unexpectedly, our structural data revealed the presence of a previously unknown subunit of the Mce1 complex, which we identified as MSMEG_3032/LucB based on our cryo-EM density map and structure-based searches of the AlphaFold database. In conclusion, our data provide structural and mechanistic insights into MCE transport systems from mycobacteria and lead to a model for Mce1-mediated fatty acid import. Our findings shed light on how the MCE systems enable pathogenic mycobacteria, such as Mtb, to scavenge resources from the host cell by providing a pathway to transport substrates across the cell envelope without compromising the protective nature of this barrier.

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Abstract 2061

Investigating the importance of lipid-binding-independent molecular interactions in membrane localization of biosensors for phosphoinositide detection

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We have previously shown that pleckstrin homology (PH) domains can bind not only to phospholipids but can also interact with proteins. Mutations in these PH domains can abolish their lipid-independent interactions while maintaining affinity for lipids. In this study we investigated the function of mutants of the phosphatidylinositol 3,4,5-trisphosphate (PIP3)-specific PH-domains of Akt and GRP1. Our aim was to compare mutant and wild-type sensors under different conditions to clarify the importance of lipid-independent interactions in the establishment of plasma membrane (PM) localization. Experiments were performed on HEK 293A cells transfected with DNA constructs containing the corresponding PH domains. At 28 h post-transfection, we investigated the basal and post-stimulation PM localization of PH domains by bioluminescence resonance energy transfer (BRET) measurements. In BRET measurements, Renilla luciferase enzyme was used as donor and Venus, a fluorescent protein targeted to the PM, was used as acceptor. EGF, insulin and vanadate were used as stimuli to induce changes in inositol lipid levels. Significant differences in the effect of mutations on PM localization were found between different mutants of Akt-PH and GRP1-PH domains. Our results showed that for Akt-PH, the basal localization of mutants differed only slightly compared to the wild type, and no significant differences were observed between Akt-PH variants after different stimuli. In contrast, for GRP1-PH mutants with retained lipid binding capacity and lacking protein binding capacity, a significant reduction in PM localization was observed compared to the wild type, both at rest and after stimulation by all three compounds. By generating sensors that contain the mutant PH-domains in tandem we managed to create biosensors that can detect changes of PM PIP3, and therefore can be used as more reliable inositol lipid probes.

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Abstract 2069

ATP-citrate lyase regulates lipid biosynthesis in *Yarrowia lipolytica*

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ATP citrate lyase (ACL) catalyzes the ATP-dependent conversion of cytosolic citrate to the fatty acid precursor, acetyl-CoA. ACL presence in yeasts has been associated with their ability to accumulate lipids (i.e., oleaginous phenotype), but little is known about the regulation of this enzyme in oleaginous yeasts. In the model oleaginous yeast *Yarrowia lipolytica*, ACL is a heterodimer composed of a catalytic and a regulatory subunit encoded by the *ACL1* and *ACL2* genes, respectively. To better understand the contribution of each of the subunits of ACL in lipid biosynthesis, we constructed strains that lack (i.e., *acl1Δ; acl2Δ; acl1Δacl2Δ*) or overexpress (OE) (i.e., *ACL*1OE; *ACL*2OE; *ACL*1/2OE) Acl1 and Acl2, either alone or in combination. The expression of ACL in the strains was confirmed by immunoblot analysis using antibodies directed against its two subunits (i.e., Acl1 and Acl2). We examined the time-dependent expressions of Acl1 and Acl2 and found that their levels decline over time. The expression profiles of Acl1 and Acl2 showed that both subunits were expressed at 24 h and 48 h, but their expression declined at 72 h and 96 h of growth. The decline in expression could indicate decreased expression of the *ACL*1 and *ACL*2 genes or degradation of the proteins. Overexpression of Acl1 increased the protein levels of Acl2. In addition, Acl2 was more expressed than Acl1 in the strain overexpressing Acl1 and Acl2. We also examined the effects of the ACL mutations on growth and lipid biosynthesis on high glucose media that induce lipid accumulation. The loss of Acl1 or Acl2 resulted in slower growth compared to the wild type, while ACL overexpression did not significantly affect growth. Lipid analysis showed that triacylglycerol levels decreased by 74% in the *acl*1Δ mutant and increased by 35% in the *ACL*1/2 OE strains compared to the wild type. The phospholipid levels decreased by 19% in the *acl*1Δ mutant and 28% in the *ACL*1/2 OE strains. The ACL mutations also affected the fatty acid profiles of the triacylglycerols and phospholipids. These effects are being further examined using an integrated omics approach.

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Abstract 2117

A short-chain acyl-CoA synthetase that supports branched-chain fatty acid synthesis in *Staphylococcus aureus*

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Staphylococcus aureus produces a combination of branched and straight chain fatty acids to maintain membrane homeostasis. Branched-chain ketoacid dehydrogenase (Bkd) is the enzyme responsible for the formation of branched-chain acyl-CoA primers from α-keto acids. 3-ketoacyl-ACP synthase III (FabH) then catalyzes the initial condensation step in fatty acid biosynthesis drawing on the available short-chain acyl-CoA primer pool. Most experiments with *S. aureus* are carried out in rich media that contains an abundant supply of branched-chain amino acids. Under these laboratory conditions isoleucine is the favored substrate for the IlvE transaminase and Bkd produces 2-methylbutyryl-CoA for anteiso fatty acid synthesis. In bkd knockout strains, however, branched-chain fatty acids are still present and the enzyme(s) responsible for this alternate biosynthetic pathway is unknown. Gas chromatography analysis of the fatty acid composition of bkd knockout strains grown in rich media shows an increase in iso even fatty acid chains and a decrease in iso odd chains. The anteiso branched-chain fatty acid composition is elevated by supplementation with 2-methylbutyrate suggesting a pathway that activates short-chain acids to their acyl-CoAs. The bkd knockout strains are auxotrophs for either 2-methylbutyric acid or isobutyric acid in defined media supporting the activation pathway hypothesis. A screen of candidate genes identified the mbcS gene (methylbutyryl-CoA synthetase; SAUSA300_2542) as the first required step in the incorporation of 2-methylbutyric and isobutyric acids into phosphatidylglycerol. MbcS is an ATP/Mg2 +-dependent acyl-CoA synthetase that selectively catalyzes the conversion of isobutyrate and 2-methylbutyrate to their respective acyl-CoA. Butyrate and isovalerate are poor MbcS substrates and activity was not detected using acetate or short-chain dicarboxylic acids as substrates. Thus, MbcS functions as a salvage pathway to convert extracellular 2-methylbutyric and isobutyric acids to their respective acyl-CoAs that are used by FabH to initiate branched-chain fatty acid biosynthesis for membrane formation independent of Bkd function.

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Abstract 2151

How to build, manipulate, and destroy functional nanodomains in cell membranes

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While all membranes share a characteristic bilayer morphology, their lateral organization can be remarkably complex. In biological membranes, lipids and proteins self-assemble to generate a variety of compositionally distinct domains that range in size from nanometers to microns, exist over a wide range of time scales, and assume varying curvatures and morphologies. Our group seeks to understand the physicochemical principles that govern the assembly and function of two related yet distinct classes of membrane nanodomains: membrane rafts and caveolae. Both reside within the plasma membrane of cells, form in a cholesterol-dependent manner, regulate multiple cellular processes, and, when defective, contribute to human disease. Yet, they differ substantially in morphology, lifetime, and function. How these two discrete classes of domains accomplish the many tasks ascribed to them remains a fundamental unanswered question in biology. Here, I will summarize lessons we have learned using biophysical approaches about the connections between the structure, dynamics, and function of these enigmatic nanodomains. I will also present recent insights emerging from our work on two major questions in the field: 1) What is the structural basis for caveolae formation? and 2) Are rafts druggable targets?.

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Abstract 2153

Effects of PTH1R ECL1 mutants on Ca2+ sensing and intracellular G protein binding

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Parathyroid hormone 1 receptor (PTH1R) is a family B G protein coupled receptor (GPCR) that, with its two ligands parathyroid hormone (PTH) and parathyroid hormone related protein (PTHrP), is responsible for maintaining bone homeostasis, calcium regulation and bone remodeling. PTH1R shows prolonged ligand binding and activation for PTH, but not PTHrP, when extracellular calcium is present. Mutating non-conserved, negatively charged residues in PTH and the extracellular loop 1 (ECL1) of PTH1R removed the calcium sensing ability observed in ligand binding assays. However, very little is known about the effect of PTH calcium sensing on intracellular G protein interactions with PTH1R. Here, we test ECL1 mutant PTH1R to investigate the role of these residues in potential interactions with calcium and how they change the intracellular interaction between PTH1R and difference G protein isoforms. We mutated acidic residues of the PTH1R ECL1 to alanine and incorporated the mutant constructs into FRET based SPASM (systematic protein affinity strength modulation) sensors. PTH1R SPASM sensors contain PTH1R, the acceptor fluorophore, a flexible ER/K linker, the donor fluorophore, and a G peptide, which mimics the interacting domain of the full G protein. PTH binding activates PTH1R, which interacts with a G peptide, bringing the donor and acceptor fluorophores into close proximity and increases the FRET ratio. Changes in FRET ratio after PTH activation of WT PTH1R varies depending on the G peptide isoform. Mutant PTH1R SPASM sensors show distinct G peptide interactions compared to WT PTH1R SPASM sensors. Further, the presence of calcium affects the mutant PTH1R SPASM differently than the WT PTH1R SPASM sensors. This data provides a crucial link between the PTH calcium sensing measured in extracellular ligand binding assay and changes in intracellular G protein interactions. Understanding the role of extracellular calcium sensing in PTH1R activation and signaling will give way to more targeted drug development for diseases that affect bone remodeling and calcium homeostasis.

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Abstract 2154

Evidence of metabolite binding to the hydrophobic clefts of perilipins 3 and 5

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John Tansey

Obesity, type II diabetes mellitus, and non-alcoholic fatty liver disease are all diseases associated with dysfunction of lipid metabolism or lipolysis– the breakdown of lipids into free fatty acids and glycerol. Neutral lipids in the cell are stored in lipid storage droplets coated with at least one of the perilipin family of proteins, essential regulators of lipase function. Previous work by Hickenbottom et al. has shown that the carboxy terminus of perilipin 3 folds into a boot-like structure consisting of a four-helix bundle capped by a mixed α-β domain. At the interface of the four-helix bundle and the mixed α-β domain is a hydrophobic cleft of unknown function. The genomic organization, nucleotide sequence, and amino acid sequence of perilipins 3 and 5 are highly conserved, particularly in the region that contains this hydrophobic cleft. Threading the sequence of perilipin 5 onto perilipin 3 using both RAPTOR X and PHYRE 2 produces a similar structure that also includes conservation of the pocket of interest. We hypothesized that this cleft could bind to hydrophobic metabolites and affect the function of these proteins. To study interactions of the hydrophobic pocket, models of the proteins were docked in silico to a series of small organic biological molecules using the GOLD software package from Cambridge Crystallographic Database. Mono and bicyclic systems were docked and yielded high CHEMPLP scores ranging from approximately 25 to 45. Further, a series of fatty acids with varying carbon chain lengths and degrees of unsaturation were docked and yielded high scores ranging from approximately 40 to 82 in perilipin 3 with similar scores for the threaded structure of perilipin 5. Increasing CHEMPLP scores correlated with an increase in carbon chain length but did not correlate with degree of unsaturation. The highest scores produced in the threaded structure of perilipin 5 were obtained by docking a di- or triglyceride. Docking results were visualized and fits verified and compared using the molecular visualization software HERMES. The increasing CHEMPLP scores for the molecules examined, and their fits into the pocket seen in Hermes, indicate that a hydrophobic metabolite may bind to this pocket, potentially impacting the biological function of this class of proteins. Additionally, this work may provide a means for the development of small molecules which could be used as possible pharmacological interventions for the treatment of lipid storage disorders.

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Abstract 2163

Discovering the Functional Role of Plasma Ceramides in Adaptive Thermogenesis

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Judith Simcox

Lipids in the blood plasma facilitate inter-organ communication by functioning as signaling molecules to inform on energy availability and inflammatory state. Most lipids in the plasma are functionally uncharacterized, and our lab uses the physiological stress of cold exposure to rapidly shift plasma lipid abundance and determine lipid function. In cold exposure, plasma lipids produced by peripheral tissues are required to fuel and activate heat production (thermogenesis) in brown adipose tissue. One of the lipid classes that is increased with cold exposure is plasma ceramides. Within 6 hours of cold exposure, plasma ceramides containing very long acyl chains are increased by two-fold. To determine if these plasma ceramides function to regulate body temperature, we used myriocin, a drug that inhibits ceramide synthesis. Myriocin treatment for one hour before cold exposure ablated the cold-induced rise in plasma ceramides and caused cold intolerance. These results suggest that plasma ceramides are important for thermogenesis. To determine the functional role of plasma ceramides in thermogenesis, we treated brown adipocytes with ceramides. Because ceramides are bound to high density lipoprotein (HDL) in cold exposure, we HDL-conjugated these ceramides and measured the impact on brown adipocyte function through assessment of mitochondrial respiration. Ceramides increased brown adipocyte respiration in response to a cold exposure mimetic, β3-adrenergic receptor agonist. Our future studies will aim to determine how these plasma ceramides are functioning to increase brown adipocyte respiration. In other conditions where plasma ceramides are elevated, such as type 2 diabetes, ceramides have been shown to signal for inflammation, cell proliferation, and insulin responsiveness. We will begin by exploring if these established signaling roles are contributing to the ability of ceramides to regulate body temperature. This work will identify molecular mechanisms governing ceramide function in the mammalian response to cold and better our understanding of the systemic lipid metabolism dysregulated in disease.

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Abstract 2165

Role of PTH Ca2+ sensing residues on GGa protein interactions with binding to PTH1R

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Parathyroid hormone 1 receptor (PTH1R) is a family B GPCR that plays a crucial role in bone remodeling. Previous studies show Past studies demonstrated that extracellular Ca2+ acts asis a positive allosteric modulator for one of PTH1R ligand, parathyroid hormone (PTH), which is approved by US FDA to treat severe osteoporosis. Moreover, PTH residues E19 & E22 have shown to involved in Ca2+ sensing. However, the effects of PTH Ca2+ sensing on intracellular G protein binding are unknown. Here, we use FRET based SPASM sensors to study the interaction between PTH1R and different Ga peptides. SPASM sensors are constructed withsensors, which are isolated in native HEK293T cell membranes through optimized membrane preparation protocol, contain PTH1R followed by the acceptor fluorophore, a flexible linker, the donor and acceptor fluorophore and a peptide from as, with different activation light wavelength, that are connected via a linker and attached to various Ga subunit that mimics the interaction of the full G protein heterotrimerisoforms or C terminal tail of the PTHR1,. respectively. After specific excitation, the emission of the donor fluorophore will excite the acceptor fluorophore when the two are in close proximity. Thus, the FRET output indicates the distance between Ga peptide and PTH1R. We performed FRET experiments quantifiedto quantify the activation. FRET ratios were used to indicate changes in activation of different Ga isoforms by PTH and its calcium sensing mutant, PTHE19AE22A. PTH binding to PTH1R SPASM sensors causes differential interactions between PTH1R and the Gs, Gq and Gi peptides. PTHE19AE22A activation of PTH1R SPASM sensors leads to distinct interaction profiles between each G peptide isoforms, which were further modulated by the presence of extracellular Ca2+. Quantifying the Current preliminary data suggest distinct interaction profile between each Ga peptides and PTH1R upon extracellular PTH and PTHE19AE22A activation. Potential change in intracellular binding interaction in the presence of Ca2+ was also observed. when PTH or mutant PTHE19AE22A stimulate SPASM sensor. Exploring dDifferential activation of specific Ga isoforms by PTH and PTHE19AE22A in the presence and absence of Ca2+ the specific signaling pathways activate by PTH and its mutant will l delineate delineate mechanistic details about of PTH1R activation and its role in bone related diseases. Further, understanding the extracellular Ca2+ modulation of PTH signaling will provide insight for developing treatments for chronic hypocalcemia associated with hypoparathyroidism, while uncovering novel regulation of bone remodeling.

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Abstract 2166

Peroxisomal import determinants of a human reductase involved in plasmalogen biosynthesis

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Vanina Zaremberg

Plasmalogens are the major ether glycerophospholipid constituents in most mammalian tissues, including the brain, heart, kidney, lung, and skeletal muscle as well as mature spermatozoa. The synthesis of ether lipids begins in peroxisomes. The last peroxisomal step catalyzed by a reductase bridges the movement of ether lipid precursors to the endoplasmic reticulum where the synthesis of complex plasmalogens is finalized. This enzyme is called alkyl/acyl dihydroxyacetone-phosphate reductase (ADHAPR) and catalyzes the reduction of alkyl dihydroxyacetone-phosphate into alkyl glycerol-3-phosphate. The gene encoding ADHAPR was identified in mammals as DHRS7B based on homology with a yeast reductase Ayr1 catalyzing a similar reaction. Yeast does not possess ether lipids and Ayr1 is a lipid droplet resident protein that participates in a shunt pathway for biosynthesis of glycerolipids. In contrast, human ADHAPR (hADHAPR) is a peroxisomal membrane protein (PMP) with a single transmembrane domain that belongs to the short-chain dehydrogenases/reductases (SDR) family. We aim to characterize the peroxisomal targeting mechanism of hADHAPR. Based on previous work, we hypothesize that the N-terminus of hADHAPR is necessary and sufficient for its peroxisomal import via a Pex19-dependent mechanism. In this work we have validated the use of budding yeast *Saccharomyces cerevisiae* to tackle these studies. The human enzyme was successfully expressed in yeast and complementation assays were used to assess its functionality in cells lacking Ayr1. In addition, a catalytically dead mutant was designed by site directed mutagenesis of conserved signature motifs of the SDR family. Using live fluorescence microscopy, it was determined that hADHAPR localized to peroxisomes which were, in most cases, partially associated with lipid droplets. Localization dependence on activity was assessed. Even though peroxisomal membrane proteins do not have a conserved targeting signal (like peroxisomal matrix proteins), hADHAPR was imported to peroxisomes in yeast suggesting a generally conserved import signal for PMPs. Interestingly, overproduction of hADHAPR induced peroxisomal proliferation, an effect not seen when other peroxisomal enzymes of

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this metabolic pathway containing peroxisomal targeting signals (PTS1 and PTS2) were overproduced at similar levels. Our study contributes to better understand signature motifs required for targeting of membrane proteins to peroxisomes.

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Abstract 2173

Human skin and keratinocytes in culture synthesize sphingoid bases up to C29-carbon chain length

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Elena Goleva, Katherine Lyubchenko, Anna Sofia Bronoff, Irina Bronova, Donald YM Leung

Stratum corneum, the top layer of human skin, contains diverse ceramides that fulfill skin barrier function. It is currently known that skin ceramides contain even and odd-chain sphingoid bases with a chain-length up to C26. However, there is no full information about the chain-length diversity of free sphingoid bases present in the skin. To answer this question, we have performed a study of sphingoid bases of S- and DS-series originated from stratum corneum total lipids. Total stratum corneum lipids were subjected to acidic hydrolysis, formed free sphingoid bases were extracted with diethyl ether and partially purified using silica solid phase extraction. The neutral loss of 18 Da scan has revealed multiple ions that correspond to S-series sphingoid bases up to C26 and D-series sphingoid bases up to C29. The product ion scan of the abundant ions with the m/z 414.3 and m/z 442.3 confirmed their breakdown pattern to be identical to that of the standard C18-dihydrosphingosine with the m/z 302.2, thus suggesting their structure as C26- and C28-dihydrosphingosines, respectively. The LC-MS/MS of the intact stratum corneum lipids revealed abundant peaks of free C24- and C26-dihydrosphingosines. Interestingly, free sphingosines prevailed over dihydrosphingosines up to the chain-length of C21 in the total lipids from stratum corneum but were present only in trace amounts above C26 chain length. Finally, a stable isotope pulse-labeling experiment was performed with Ca2+-differentiated human keratinocytes and [U-13C,15N]-serine *in vitro*, and label incorporation into free dihydrosphingosines up to the chain length of C28 was observed that additionally confirmed the biosynthesis of very long sphingoid bases by differentiated human keratinocytes. Thus, our study revealed an extended ability of differentiated keratinocyte serine palmitoyltransferase to synthesize very long chain sphingoid bases with the chain length up to C29. It also suggests limited capacity of ceramide synthases to utilize dihydrosphingosines with the chain length above C22 for dihydroceramide biosynthesis.

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Abstract 2179

SIR telomere silencing depends on nuclear envelope lipids and modulates sensitivity to a lysolipid drug

Maria Laura Sosa Ponce, *University of Calgary*

Mayrene Horta Remedios, Sarah Moradi-Farf, Jennifer Cobb, Vanina Zaremberg

The nuclear envelope (NE) is important in maintaining genome organization. The role of lipids in the communication between the NE and telomere silencing was investigated, including how changes in lipid composition impact gene expression and overall nuclear architecture. For this purpose, yeast cells were treated with the non-metabolizable lysophosphatidylcholine analog edelfosine, known to accumulate at the perinuclear endoplasmic reticulum. Edelfosine treatment induced NE deformation and disrupted telomere clustering but not anchoring. In addition, the association of Sir4 at telomeres measured by ChIP decreased. RNA-seq analysis showed altered expression of Sir-dependent genes located at sub-telomeric (0–10 kb) regions, which was consistent with Sir4 dispersion. Transcriptomic analysis revealed that two lipid metabolic circuits were activated in response to edelfosine, one mediated by the membrane sensing transcription factors, Spt23/Mga2, and the other by a transcriptional repressor, Opi1. Activation of these combined transcriptional programs resulted in higher levels of unsaturated fatty acids and the formation of nuclear lipid droplets. Interestingly, cells lacking Sir proteins displayed resistance to unsaturated fatty acids and edelfosine, and this phenotype was connected to Rap1.

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Abstract 2222

Development of scalable assays for detection of PIKfyve activity

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PIKfyve is a phosphoinositide kinase that produces PI(3,5)P2 from PI(3)P and has core functions in the endosomal and lysosomal pathways. PIKfyve activity has been implicated in a wide range of disorders including some cancers, autoimmunity, and motor neuron disease. More recent interest has arisen due to the SARS-CoV-2 pandemic and the role PIKfyve plays in viral entry into host cells. While there are some small molecule PIKfyve inhibitors available, they have shown limited efficacy in the clinic despite having a favorable safety profile and being well tolerated. Thus there is still a high degree of interest in developing novel, potent inhibitors of PIKfyve to treat various diseases. One of the main barriers in development of new PIKfyve inhibitors is the available assays to assess PIKfyve activity. As with other lipid enzymes, current assays require the use of either a radioactive label or detection by mass spectrometry (MS) to obtain a readout of conversion of PI(3)P to PI(3,5)P2. This limits their utility in drug development and compound screening as they are not easily scalable. Here we describe the development of a competitive assay for PIKfyve activity that directly detects its reaction product PI(3,5)P2 without the need for radioactive labels or extraction for MS analysis.

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Abstract 2238

The role of ATG9 in Phagophore Formation During Autophagy

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The Moeller Molecular Modeling Team in conjunction with Dr. Jiajie Diao at the University of Cincinnati Medical College used 3D modeling and printing technology to study the vital role of Atg9 – the only transmembrane protein – in the cellular degradation process of autophagy. Immunofluorescence testing shows that Atg9 is recruited to the phagophore site by Atg11 and the FIP200 complex and later is embedded in the membrane of the phagophore. During autophagy, Atg9 localizes with Atg2 and Atg18 at the Endoplasmic Reticulum Exit Site (ERES). Atg9 being the only transmembrane lipid scramblase protein in autophagy is the main protein responsible for the formation of autophagosomal membranes. Atg2 receives lipids from the ER and transports them to Atg9 which then distributes the lipids across both leaflets of the membrane causing the phagophore membrane to expand. In addition, Atg9 allows for the membrane to bend creating a vacuole around misfolded proteins and ubiquitinated substrates on the FIP200 complex facilitating their removal from the cell.

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Abstract 2271

Perilipin 5 Trafficking in CHO Cells

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Metabolic diseases such as type II diabetes mellitus and non-alcoholic fatty liver disease are currently contributing to a health crisis both globally and here in the United States. Key to understanding these diseases and their progression is an understanding of neutral lipid metabolism. The perilipins are a class of five conserved proteins that are key regulators of lipid metabolism that have been implicated in lipid trafficking within cells. The most recently discovered member of the family is perilipin 5, which is expressed most strongly in tissues that are highly oxidative such as heart, oxidative muscle, and fasting liver. Perilipin 5 is predominantly located on the surface of lipid storage droplets, but has also been found in the cytoplasm, nucleus, the endoplasmic reticulum, and mitochondria. We hypothesized that perilipin 5 moves between these cellular pools via interactions with other proteins including motor proteins, cytoskeletal elements, and Rabs. Furthermore, we hypothesized that interactions with Rabs were necessary for this trafficking to occur. CHO cells expressing perilipin 5 and different Rabs were used as a model system to investigate this interaction. Immunoprecipitations were used to assay interactions between perilipin 5, Rab18, and Rab32 and positive interactions were found between perilipin 5 and Rab18. Immunofluorescence microscopy was used to identify perilipin 5 and different Rabs in the cell, and colocalization was seen between perilipin 5 and Rab18. Collectively, these data provide a more detailed picture of perilipin 5 function in the cell.

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Abstract 2301

Carboxylesterase 1 (CES1) regulates arachidonic acid-containing triacylglycerol levels in macrophages and shapes their immunophenotype

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Maggie Phillips, Abdolsamad Borazjani, Matthew Ross

Triacylglycerols (TAGs) are lipids found in cytoplasmic lipid droplets. Toll-like receptor activation causes increased TAG accumulation in macrophages and enhances their inflammatory function. The increase in TAG-containing lipid droplets in the setting of inflammation is due to both enhanced biosynthesis and reduced fatty acid release. Human carboxylesterase 1 (CES1) is a member of the serine hydrolase superfamily and catalyzes the hydrolysis of TAG lipids, including those containing oxidized polyunsaturated fatty acids. CES1 is expressed in monocytes/macrophages, although its function in these cells is unclear. When CES1 expression was stably knocked down in THP-1 macrophages, marked increases in arachidonic acid-containing TAGs was detected by LC-HRMS; ~15–20-fold higher levels than those in control THP-1 macrophages where CES1 expression was normal. An increased amount of Bodipy-labeled TAGs was also measured by confocal microscopy in the CES1 knockdown cells. In addition, the metabolic fate of 15-hydroxyeicosatetraenoic acid (15-HETE), which is the enzymatic product derived from IL-4-induced ALOX15, was found to be significantly altered in the CES1 knockdown (KD) cells. Exogenously added 15-HETE (300 nM) was rapidly metabolized in macrophages by peroxisomes to tetranor 15-HETE derivatives, or it was metabolically incorporated into the TAG pool. Interestingly, we found that the level of esterified 15-HETE in TAGs was ~7-fold higher in CES1KD cells than in control cells after a 6-hr treatment. Furthermore, both RNA-seq and RT-qPCR data indicated that there were pronounced differences in the responses of control and CES1KD macrophages to M1 and M2 inflammatory stimuli (LPS/IFNg and IL-4, respectively), with the CES1KD macrophages exhibiting a more pro-inflammatory phenotype. The observed metabolic changes in TAG/oxylipin disposition and the resulting altered immunophenotype in the CES1KD cells is likely due to the reduced TAG hydrolytic activity and subsequent buildup of cellular TAGs, which enhances inflammation.

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Abstract 2327

Detergent-free extraction and spectroscopic investigation of structural dynamics in *E. coli* ATP synthase

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ATP synthase is a membrane protein complex that synthesizes adenosine triphosphate (ATP), an integral energy-carrying molecule in all forms of life. The complex is comprised of two rotary motors, the soluble F1 sector, which catalyzes ATP synthesis, and the membrane embedded Fo sector, in which rotation of the c-ring (rotor) is driven by the electrochemical gradient of H+ across the cell membrane. Movement of H+ through Fo is facilitated by two aqueous half-channels within subunit a (stator) and at the rotor-stator interface. The mechanism of H+-driven rotation in Fo is not fully understood. Cryo-electron microscopy has revealed structures of *E. coli* F1 Fo ATP synthase, but inconsistencies between this structural data and previous cross-linking data suggest the existence of multiple conformations of the stator. To facilitate spectroscopic characterization of purified F1Fo, we used amphipathic polymers (styrene-acrylic acid or amphipol) to extract complexes from the cell membrane without introducing detergents. ATPase activity of the resulting nanodiscs was confirmed, and site-directed spin labeling and electron paramagnetic resonance (EPR) spectroscopy were used to probe backbone mobility of the stator in comparison to complexes in detergent micelles or liposomes. Optimization of these ATP synthase nanodiscs will allow spectroscopic observations to determine the conformational dynamics of the stator subunit in a native membrane environment.

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Abstract 2386

DNP-NMR as a tool for probing the regulation of PKC signaling by membrane dissolved phorbol esters

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Phorbol esters bind to C1 domain containing proteins and are potent activators of protein kinase C (PKCs). Modulators of PKC activation are a promising class of anti-cancer therapies and HIV latency reversing agents. A better understanding of the interactions between membrane components and phorbol esters is critical for understanding how ligand membrane interactions effect PKC mediated signaling. Here we uncover the local heterogeneity of 13C21,22-phorbol (PMA) within T cell membranes utilizing 13C DNP-NMR spectroscopy. We demonstrate the localization of PMA to cellular membranes and the role of cholesterol in the observed heterogeneity. The presence of cholesterol skews the chemical shift distribution downfield in both model membranes and within intact cells indicating increased head group and solvent contacts. We observe the protein bound state and can identify multiple membrane topologies within whole cells. We demonstrate the power of in-cell DNP-NMR to reveal the true scope of chemical heterogeneity of drug molecules within intact cells and provide evidence for the effect of cholesterol in phorbol ester localization.

This work was supported by the SNF project grant awarded to A.B.B and ETH Zurich internal funding.

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Abstract 2416

Modulation of plasma membrane cholesterol affects DAT function in the presence of methamphetamine

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The dopamine transporter (DAT) is essential for the reuptake of released the neurotransmitter dopamine (DA) in the brain. DAT is one of the main targets for psychostimulants leading to a disruption of DA homeostasis. There is substantial evidence that the function and plasma membrane availability of the biogenic amine transporters are influenced by cholesterol-dependent membrane nanodomains referred to as membrane rafts. To begin to understand how the psychostimulant methamphetamine (METH) affects DAT nanodomain association, we examined DAT colocalization with known membrane raft and non-raft proteins. We found that METH significantly increases the colocalization of DAT with cholesterol-dependent membrane raft marker GM1 in mouse midbrain DA neurons and significantly decreases the colocalization of DAT with the non-raft protein NA/K-ATPase. Density-gradient centrifugation revealed that DAT is distributed into both classically defined, cholesterol-dependent membrane raft and non-raft fractions. Interestingly, incubating DA neurons with METH shifted some of the DAT from non-raft to raft fractions; however, depletion of membrane cholesterol with a statin 48 hours before treatment with METH restored the distribution of DAT to that of mock treated controls. We used uptake to measure DAT function. Using high lipid serum to increase plasma membrane cholesterol in DA neurons, significantly reduced DAT uptake, while using statins to lower membrane cholesterol, significantly increased DAT uptake. As expected, treatment with METH significantly lowered DAT uptake. However, DAT uptake in DA neurons pretreated with statins before METH addition was no different from mock treated controls. These data suggest that cholesterol modulation and nanodomain association are important for activity-dependent DAT function in the presence of METH. These findings may lead to new therapies for METH addiction.

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Abstract 2424

Using martini3 and alphafold2 for understanding the structural basis of epha2-dimerization, -membrane interactions and implications of tm/jm mutations in cancer progression

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Amita Sahoo, Pravesh Shrestha

EphA2 plays a critical role in cellular growth, differentiation and motility. In line with EphA2 mRNA expression in multiple tissues and organs, its overexpression is reported in several different cancer types, and even in cancer-derived cell lines. EphA2 overexpression may have significance and could be used as a biomarker in the clinical management of cancer. Besides this, the differential EphA2 expression in normal versus cancer cells makes it a key therapeutic target. In EphA2, ligand binding regulates the monomer-dimer equilibrium through stabilization of the dimeric state by inducing a conformational change in the extracellular domain. Thus, dimerization is a key regulatory step in the activity and signaling process of EphA2. In EphA2, the juxtamembrane (JM) region follows the TM domain at one end and connects to a catalytic domain at the other. The JM domain functions in synergy with the TM domain for signal transduction. The JM domain has several basic residues (K/R) that are closely positioned to the membrane surface. Other signaling molecules specifically PIP2 and PIP3 utilize these basic amino acids for binding and occlude the nearby region from phosphorylation. Therefore, studying the structural mechanism of EphA2 dimerization and membrane interactions will help us better understand the activity and signaling of this receptor in different cancer types. Importantly, working with membrane proteins is extremely challenging. However, the recent developments in molecular modeling (Alphafold2) and dynamics simulation (coarse grained Martini3) have provided a powerful tool to study membrane proteins and their interactions. Here, we modeled the relevant domains of the EphA2 receptor and studying its structural mechanism of activation for signaling by harnessing the power of advanced in-silico techniques. We find that while there may be specific interactions between the EphA2 fibronectin domains which are just outside the cell, the TM dimers and JM association is essentially insensitive to most of the cancer associated mutations.

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Abstract 2465

Understanding the role of glycosphingolipids in membrane organization of the yeast vacuole

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Itay Budin

When yeast transitions from exponential to stationary growth phase, yeast actively tunes its vacuole membrane to form membrane domains that are known as lipid rafts. These domains allow the docking of lipid droplets to the vacuole for lipophagy under starvation conditions. Sphingolipids have been implicated as one of the major components of lipid rafts, so we investigated the role of sphingolipids in vacuole domain formation. *Saccharomyces cerevisiae* can biosynthesize three types of sphingolipids, which are all glycosylated. Lipidomics analysis of biochemically isolated stationary phase vacuoles revealed a dramatic increase (>2-fold) in total glycosphingolipids (GSLs), especially inositol phosphorylcearmide (IPC), compared to exponential phase vacuoles. IPC is the simplest GSL present in yeast. This change was not present in whole cell lipidomes of the same cells, indicating that GSLs get sorted into the vacuole during stationary growth phase. To test the role of different GSLs in vacuole phase separation, we systematically engineered glycosphingolipid composition in a series of strains. Reduction of IPC levels through modulation of AUR1 expression strongly inhibited domain formation. Confocal microscopy showed that vacuole domain morphologies are altered in cells lacking more complex GSLs compared to wild-type cells. Our results show that the metabolism and trafficking of GSLs is a key driver of membrane phase separation in yeast vacuole.

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Abstract 2471

Dissecting the role of sterol structure to lipid domain formation in living cells

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The Bloch hypothesis states that sterols have evolved to support optimized cellular and physiological functions. In the context of cholesterol biosynthesis, the product of each step has an improved ordering ability compared with its precursors. Here we apply Bloch's model in the yeast vacuole, an *in vivo* system where ordered lipid domains (rafts) act as lipid droplet docking sites for lipophagy under starvation conditions. Through genetic perturbations of the ergosterol biosynthesis pathway, we drive the accumulation of different ergosterol intermediates in cells. Employing fluorescence microscopy, we quantified changes in vacuole raft formation as a function of sterol species in stationary cells with the aid of well-established raft associated protein marker Pho8-GFP. Interestingly, we observed a non-linear trend, where accumulation of early stage intermediates drove raft formation, while raft formation is disrupted in late stage intermediates relative to the final product (ergosterol). Analogous experiments in model lipid membranes corroborated our findings. These results suggest a complex evolutionary trajectory for ergosterol biosynthesis, where small structural differences in late stage intermediates do not predictably enhance raft formation, contrary to the Bloch hypothesis. Thus, ergosterol serves as a consensus sterol in which raft formation is one of several functions lipid evolution selected for.

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Abstract 2486

How lipid chemistry shapes biology: from the powerhouse of our cells to the bottom of the earth's oceans

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Small changes in the structure of lipids can have dramatic effects on membrane properties and dynamics membranes in cells. I will present two stories that use lipidome dissection to uncover mechanisms by which key membrane structures are generated and maintained under different environmental conditions. In the first, we have elucidated how the highly curved morphology of the mitochondrial inner membrane is governed by the mechanical interactions between unsaturated phospholipid acyl and cardiolipin, a unique conical lipid synthesized in the mitochondria. In the second, we have discovered a class of ether phospholipids that allows deep sea animals to withstand the crushing effects of pressure on lipid bilayers, but also structurally constrains their survival to extreme depths. These projects both highlight how homeostatic maintenance of lipid shape could serve as a general mechanism underlying differences in the chemical composition of cell membranes.

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Abstract 2515

A proposed role for VPS13 and related proteins in bulk lipid transfer and membrane expansion

Karin Reinisch, *Yale*

VPS13 proteins, conserved in all eukaryotes from fungi through humans, are of significant biomedical interest because their dysfunction in humans is associated with severe neurodegenerative diseases, including Parkinson's disease for VPS13C. Recently, VPS13s as well as members of the VPS13-superfamily such as ATG2, are proposed to function as bridges that allow for directional bulk lipid transfer between organellar membranes at contact sites. Emerging is the notion that they transfer lipids for membrane expansion and organelle biogenesis processes, many of which were until recently believed to rely exclusively on vesicle fusion. For example, the lipid transfer ability of yeast Vps13 is required for the growth of the prospore membrane, and ATG2 lipid transfer ability is prerequisite for autophagosome biogenesis. In their overall architecture, these proteins resemble elongated taco shells, featuring a long hydrophobic groove to solubilize lipid fatty acid moieties as lipids travel along the bridge across the cytosolic space between organellar membranes. Recent studies suggest that the proteins function as components of lipid transport systems. These systems include adaptor proteins that interact with the taco shell ends to anchor VPS13-superfamily proteins between organelles and they include scramblases in the donor and/or acceptor organelle membranes. Scramblases are proposed as required for protein-mediated bulk lipid transfer because they re-equilibrate lipids between bilayer leaflets as lipids are transferred between cytosolic leaflets only of apposed membranes, thus preventing build-up of energetically costly membrane bilayer asymmetry.

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Abstract 2529

CryoEM Yields New Insights into Lipoprotein Lipase Structure and Function

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Kathryn Gunn

Lipoprotein lipase (LPL) is an enzyme that hydrolyzes triglycerides from circulating lipoproteins such as VLDL and chylomicrons to release free fatty acids for use by underlying tissues. Using cryogenic electron microscopy (cryoEM), we determined two unique structures of LPL. The first structure is an active LPL dimer resolved to 3.9 Å resolution. This structure show that the lipase has undergone a rearrangement of amino acids to open a hydrophobic pore passing through the protein adjacent to the active site. Modeling a free fatty acid into the pore suggests how product can be released following substrate hydrolysis. Additionally, this new structure features an unexpected dimerization interface. We also present a cryoEM structure of LPL helices. LPL in helices is inactive. The elucidation these novel LPL structure highlights how it can adopt a diverse range of quaternary structure as it travels from secretory vesicles in the cell, to the capillary, and eventually to the liver for lipoprotein remnant uptake.

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Abstract 2548

Responsive assembly of signaling domains at the plasma membrane

Sarah Veatch, *University of Michigan*

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Plasma membrane heterogeneity has been tied to a litany of cellular functions and is often explained by analogy to membrane phase separation. We present an updated model of plasma membrane heterogeneity in which the plasma membrane is a highly adaptable fluid, capable of adjusting its local composition in response to protein scaffolds. This model is rooted in experiments and theory first explored in model membranes, and is extended to intact cell membranes through quantitative super-resolution nanoscopy measurements in live B lymphocytes that detect functional membrane domains that emerge upon clustering B cell receptors. The membrane phase transition can also augment interactions between proteins that make up these scaffolds, which sometimes take the form of protein-rich liquid droplets, influencing their assembly and stability at the membrane surface. This presentation will highlight current theoretical and experimental support for this emerging picture of plasma membrane organization and function.

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Abstract 2549

Lipid-dependent assembly and budding of emerging viral pathogens

Robert Stahelin, *Purdue University*

Lipid enveloped viruses replicate and bud from the host cell where they acquire their lipid coat. Lipid-enveloped viruses include dangerous pathogens such as coronaviruses (SARS-CoV-2, etc.), filoviruses (Ebola virus and Marburg virus) and paramyxoviruses (Nipah virus, Hendra virus, etc.). Despite understanding some of the basics of how these viruses cause disease and enter host cells, not much is known on how these dangerous pathogens interact with host cell lipids to achieve new virion formation. The viral matrix or membrane protein regulates assembly and budding from the host cell membrane, connecting the viral lipid envelope to the viral nucleocapsid. Depending on the virus family, this assembly and budding may occur at the plasma membrane or the ER-Golgi intermediate compartment. This presentation will detail the biophysical and biochemical basis of how these emerging pathogens hijack host lipid membrane and metabolic networks to form new virus particles that undergo release from the host cell.

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Abstract 2552

Reversible, large-scale, liquid-liquid phase separation in living yeast membranes

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Chantelle Leveille, John Reinhard, Caitlin Cornell, Glennis Rayermann, Scott Rayermann, Christian Klose, Robert Ernst, Alexey Merz

Liquid-liquid phase separation in living biological membranes is usually described as occurring on sub-micron length scales. A stunning counterexample occurs in *S. cerevisiae*. When the yeast shift from the log stage of growth to the stationary stage, huge, micron-scale liquid domains appear in the membranes of the vacuole, an endosomal organelle. These phases are functionally important, enabling yeast survival during periods of stress. Here, we investigate yeast vacuoles using fluorescence microscopy and lipidomic techniques. This talk will review results showing: (1) Miscibility transitions in yeast vacuole membranes are reversible as would be expected from equilibrium thermodynamics, even though the transitions occur in a living system. (2) Yeast actively regulate this membrane phase transition to occur ~15C above the yeast growth temperature. (3) Yeast significantly remodel their vacuole lipidomes in the shift from the log stage to the stationary stage.

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Abstract 2554

Effect of Maternal Body Mass Index and Gestational Age on Placental Lipid Profiles in Early Pregnancy

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Introduction: The placenta maximizes fatty acid transport to promote fetal growth throughout gestation. Maternal obesity (pre-pregnancy body mass index (BMI) >30 kg/m2) has been shown to modify placental lipid metabolism in late pregnancy, promoting a lipotoxic environment with downstream effects on neonatal fat deposition. These processes have not yet been studied in early pregnancy (EP). The purpose of this study was to determine the effects of maternal BMI and estimated gestational age (EGA) on placental lipid profiles in EP.

Methods: A cross-sectional study was performed on healthy women who had previously chosen to terminate their pregnancies. Lipids from thirty-two first trimester placentas were extracted using the Folch Method. Using thin layer chromatography, lipids were identified and quantified with fatty acid standards including triglyceride (TG), free fatty acid (FFA), cholesterol ester (CE), free cholesterol (FC), phosphatidylcholine (PC), phosphatidylinositol (PI), cardiolipin (CL), and phosphatidylethanolamine (PE). Correlations between placental lipids and maternal demographic data were analyzed using non-parametric Spearman correlation ($p < 0.05$ was considered statistically significant).

Results: The average BMI of the cohort was 26.5 kg/m2, and the range of EGA was 7.1–14.3 weeks at the time of procedure based on ultrasound. Strong associations were found between total lipids and CE ($r = 0.38$, $P = 0.03$), TG ($r = 0.47$, $P = 0.006$), FC ($r = -0.53$, $P = 0.002$), and CL ($r = 0.36$, $P = 0.04$). Gestational age showed strong correlations with total lipid content ($r = 0.55$, $P = 0.001$), as well as isolated CE ($r = 0.37$, $P = 0.04$), TG ($r = 0.42$, $P = 0.02$), and FC ($r = -0.69$, $P < 0.0001$). There was no significant correlation between EP BMI and total lipid content ($r = -0.05$, $P = 0.80$). Smoking was strongly associated with PC ($r = -0.64$, $P < 0.001$) and CE ($r = 0.39$, $P = 0.03$).

Conclusion: Unexpectedly, maternal BMI was not significantly correlated to placental lipid content. These findings suggest that the downstream consequences of maternal obesity may not be apparent as early as the first trimester. 36% of women in this cohort were obese, reflective of the 40% of pregnant women who have obesity in the United States. The positive associations between EGA and lipid content are consistent with an increase in fatty acid transport to the growing fetus. This is also consistent with biological mechanisms as CL synthesis stimulates cholesterol esterification, and CE facilitates lipoprotein transport of TG in the placenta. Furthermore, inverse ratios of CE and FC may drive progesterone production by the placenta during the placental-luteal shift

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in early EP. These findings propose a critical period in the first trimester at which placental lipids are upregulated to optimize fatty acid transport capacity to the developing fetus.

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Abstract 2576

Structure and dynamics of human perilipin 3 membrane association

Michael Airola, *Stony Brook University*

Lipid droplets (LDs) are dynamic organelles that contain an oil core mainly composed of triglycerides (TAG) that is surrounded by a phospholipid monolayer and LD-associated proteins called perilipins (PLINs). During LD biogenesis, perilipin 3 (PLIN3) is recruited to nascent LDs as they emerge from the endoplasmic reticulum. Here, we analyzed how lipid composition affects PLIN3 recruitment to membrane bilayers and LDs, and the structural changes that occur upon membrane binding. We found the TAG precursors phosphatidic acid and diacylglycerol (DAG) recruit PLIN3 to membrane bilayers and define an expanded PAT domain that preferentially binds DAG enriched membranes. Membrane binding induces a disorder/order transition of alpha helices within the PAT domain and 11-mer repeats, with intramolecular distance measurements consistent with the expanded PAT domain adopting a triangular tertiary structure. In cells, PLIN3 is recruited to DAG enriched ER membranes, and this requires both the PAT domain and 11-mer repeats. This provides molecular details of PLIN3 recruitment to nascent LDs and identifies a function of the PAT domain of PLIN3 in DAG binding.

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Abstract 2588

Cholesterol esters packaging in the endoplasmic reticulum

Abdou Rachid Thiam, *CNRS*

Cholesterol homeostasis is critical for cell health and its deregulation correlates with the occurrence of several diseases, ranging from cancer to neurodegeneration or atherosclerosis. One pathway through which cholesterol levels are balanced is via its esterification in the endoplasmic reticulum (ER) by sterol O-acyltransferase enzymes. This reaction leads to the biosynthesis of cholesterol ester, a neutral lipid that is subsequently packaged into lipid droplets: organelles of lipid storage. How cholesterol ester is packaged in the ER into droplets differs from triacylglycerol, which is the most abundant neutral lipid. In my presentation, I will talk about the findings we have made on how the physical characteristics of cholesterol ester impact the way it is packaged and how that alters protein location. Our findings offer a new understanding of cholesterol storage and provide new insights into the impact of cholesterol ester on membrane biology.

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Abstract 2594

SREBPs in the control of intestinal homeostasis and tumorigenesis

Luke Engelking, *UT-Southwestern Medical Center*

Lipids are key regulators of the growth and differentiation of intestinal epithelial cells, including intestinal stem cells (ISC). High-fat diets and obesity are serious risk factors for gastrointestinal cancers in humans. Sterol regulatory element-binding proteins (SREBPs) are transcription factors that control lipid synthesis and uptake in the intestine. Our recent studies have shown that SREBPs and their major products, sterols (from SREBP-2) and unsaturated fatty acids (from SREBP-1) have a critical role in sustaining growth of the intestinal epithelia: (1) The loss of Scap, which controls intracellular cleavage events required for SREBP activation, reduces lipid synthesis and causes a lethal intestinal injury in mice characterized by a loss of intestinal crypts, which house ISCs. (2) When SREBP-2 is inactivated in intestinal epithelium, hyperplasia of intestinal crypts and hypertrophy of the intestine results. In SREBP-2 knockouts, sterol synthesis falls while unsaturated fatty acid synthesis does not, owing to an increase in SREBP-1. (3) Despite this overgrowth phenotype, ablation of SREBP-2 in intestinal epithelia of mice blocks the formation of adenomas caused by mutant APC. These findings indicate that SREBPs govern intestinal epithelial homeostasis in complex ways. SREBPs maintain homeostasis of intestinal epithelia by producing specific, yet-to-be identified lipid metabolites that sustain the proliferation of ISCs. These pathways control intestinal organ size and malignant potential, which bears on human diseases of under- or over-proliferation of the intestine, such as short gut syndrome and colorectal cancer, the 3rd most common cause of cancer death in the U.S.

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Abstract 2645

Investigation of Phosphatidylserine-Transporting Activity of Human TMEM16C Isoforms

Byoung-Cheol Lee, *Korea Brain Research Institute*

Eunyoung Kim

Lipid scrambling is a rapid process that dissipates the asymmetrical distribution of phospholipids in the plasma membrane. It is involved in various physiological functions such as blood coagulation and apoptosis. Many TMEM16 members are recognized as Ca2+-activated phospholipid scramblases, which transport phospholipids between the two leaflets of the plasma membrane nonspecifically and bidirectionally; among these, TMEM16C is abundant in the brain, especially in neuronal cells. We investigated the scrambling activity of three human TMEM16C isoforms with different N-terminus lengths. After optimizing conditions to minimize endogenous scrambling activity, an annexin V-based imaging assay was used to detect phosphatidylserine (PS) scrambling in 293T cells. Unlike previous results, our data showed that human TMEM16C isoform 1 and isoform 3 exposed PS to the cell surface. A surface biotinylation assay showed that the surface expression of isoform 2, which did not show scrambling activity, was ~5 times lower than the other isoforms. In contrast to other TMEM16 proteins, flux assays and electrophysiology recording showed TMEM16C does not possess ion-transporting activity. We conclude that the N-terminus of TMEM16C determines whether TMEM16C can translocate to the plasma membrane and facilitate scrambling activity; membrane-localized TMEM16C isoforms 1 and 3 transport PS to the outer leaflet.

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Abstract 2674

Mechanisms controlling membrane recruitment and activation of autoinhibited SHIP1

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Grace Waddell, Emma Drew, Henry Rupp

Signal transduction downstream of growth factor and immune receptor activation relies on the production of phosphatidylinositol-(3,4,5)-trisphosphate (PI(3,4,5)P3) lipids by phosphoinositide-3-kinase (PI3K). Regulating the strength and duration of PI3K signaling in immune cells, Src homology 2 domain-containing inositol 5-phosphatase 1 (SHIP1) controls the dephosphorylation of PI(3,4,5)P3 to generate PI(3,4)P2. Although SHIP1 has been shown to regulate neutrophil chemotaxis, B-cell signaling, and cortical oscillations in mast cells, the role that lipid and protein interactions serve in controlling SHIP1 membrane recruitment and activity remains unclear. Using single molecule TIRF microscopy, we directly visualized membrane recruitment and activation of SHIP1 on supported lipid bilayers and the cellular plasma membrane. We find that SHIP1's membrane localization is insensitive to dynamic changes in PI(3,4,5)P3 both *in vitro* and *in vivo*. Very transient SHIP1 membrane interactions were detected only when membranes contained a combination of phosphatidylserine (PS) and PI(3,4,5)P3 lipids. Robust SHIP1 membrane localization and relief of autoinhibition can be achieved through interactions with immunoreceptor derived phosphopeptides presented either in solution or conjugated to supported membranes. Molecular dissection of full-length SHIP1 revealed that the N-terminal SH2 domain plays a critical role in suppressing phosphatase activity. Overall, this work provides new mechanistic insight concerning the dynamic interplay between lipid binding specificity, protein-protein interactions, and activation of autoinhibited SHIP1.

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Abstract 2690

Mechanism of membrane binding in type I phosphatidylinositol phosphate kinases

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Scott Hansen

The ability for cells to establish and maintain the asymmetric distribution of peripheral membrane binding proteins is critical for signal transduction in eukaryotes. Ubiquitously important in these signaling processes are the phosphatidylinositol phosphate (PIP) lipids, which are dynamically modified by PIP lipid modifying enzymes across all intracellular membranes. Functioning primarily at the plasma membrane, type I phosphatidylinositol-4-phosphate 5-kinase (PIP5K) catalyzes the phosphorylation of PI(4)P to generate the majority of PI(4,5)P2 lipids found in cells. Recently, we determined that PIP5K exhibits a positive feedback loop based on membrane-mediated dimerization and cooperative binding to its product, PI(4,5)P2. This project aims to determine the site of PIP recognition and determine how PI(4,5)P2 binding regulates PIP5K membrane association and catalysis. Using a combination of single molecule TIRF microscopy and kinetic analysis of PIP lipid phosphorylation, we map the sequence of steps leading to productive PIP lipid binding by PIP5K. We found that the specificity loop regulates the association rate of PIP5K and helps orient the kinase to associate with PIP lipids more effectively. Once it's correctly oriented, PIP5K transitions to binding the PIP lipids in a structural motif called the PIP binding motif (PIPBM). Previously, it was believed that the PIPBM was solely able to interact with PIP5K's substrate, however our data revealed that this motif is able to interact with a variety of PIP lipids with varying strengths that correlates with the number of phosphates covalently attached to the inositol head group. We propose a two-step membrane binding model where the specificity loop and PIPBM act in unison to orient the kinase and productively engage negatively charged lipids.

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Abstract 2732

Allosteric regulation of thioesterase superfamily member 1 by a lipid sensor domain binding fatty acids and lysophosphatidylcholine

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Significance: Brown adipose tissue (BAT) burns fat to maintain body temperature through thermogenesis in mammals. Because heat production requires metabolizing large amounts of calories, increasing the activity of the tissue could treat obesity and related metabolic disorders. Recently, thioesterase superfamily member 1 (Them1) was identified to suppress thermogenesis, whereas deletion of Them1 enhanced the ability of BAT to burn fat and prevented diet-induced obesity. In this study, we show Them1 contains a lipid sensor that binds to fatty acids and lysophosphatidylcholine, which inversely regulate the activity of Them1. This lipid sensor allows for allosteric control of Them1 activity to regulate the burning of fat. Additionally, this study affirms a promising drug target to treat obesity and related disorders. Abstract: Nonshivering thermogenesis occurs in brown adipose tissue to generate heat in response to cold ambient temperatures. Thioesterase superfamily member 1 (Them1) is transcriptionally up-regulated in brown adipose tissue upon exposure to the cold and suppresses thermogenesis in order to conserve energy reserves. It hydrolyzes long-chain fatty acyl-CoAs that are derived from lipid droplets, preventing their use as fuel for thermogenesis. In addition to its enzymatic domains, Them1 contains a C-terminal StAR-related lipid transfer (START) domain with unknown ligand or function. By complementary biophysical approaches, we show that the START domain binds to long-chain fatty acids, products of Them1's enzymatic reaction, as well as lysophosphatidylcholine (LPC), lipids shown to activate thermogenesis in brown adipocytes. Certain fatty acids stabilize the START domain and allosterically enhance Them1 catalysis of acyl-CoA, whereas 18:1 LPC destabilizes and inhibits activity, which we verify in cell culture. Additionally, we demonstrate that the START domain functions to localize Them1 near lipid droplets. These findings define the role of the START domain as a lipid sensor that allosterically regulates Them1 activity and spatially localizes it in proximity to the lipid droplet.

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ASBMB

Abstract 2744

Enveloped viral lipids can impact virion infectivity

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Several enveloped viruses can enter cells through a process termed apoptotic mimicry, where the virus hijacks the cellular system used to clear apoptotic debris. The phosphatidylserine within the enveloped virus membrane binds to PS receptors, triggering engulfment. To explore how the levels of phosphatidylserine (PS) in the envelope affect virion infectivity, we produced virus in cells lacking either flippases or scramblases making particles with high and low levels of phosphatidylserine, respectively. Chikungunya virus particles high in PS were more infectious on Vero cells than particles that were PS low. However, both high and low PS particles were equally infectious on HAP1, NIH3T3 and mosquito cell cultures, suggesting PS levels can alter infectivity into cells when using PS receptors for entry but do not play a role when entering cells through other pathways. Recombinant vesicular stomatitis virus containing the Ebola virus glycoprotein were also less infectious when particles were PS low.

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Topic Category Metabolism and Bioenergetics

Abstract 160

The Consequences of Misplaced Electrons: Dissection of Human Citrin Deficiency, a Disorder of the Malate-Aspartate Shuttle

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Nour Emwas

Citrin Deficiency (CD) is a rare human disease caused by mutation of SLC25A13, which encodes a calcium-dependent aspartate-glutamate transporter that is located to the mitochondrial inner membrane (MIM). This transporter is a component of a system that utilizes malate dehydrogenase and aspartate aminotransferase isozymes on either side of the MIM to move electrons from cytosolic NADH to the mitochondrial matrix. Infants with CD present with a bile elimination disorder and what could be mistaken for signs of a primary urea cycle and/or mitochondrial disease. Urea cycle disfunction can be explained by a lack of cytosolic aspartate. Other aspects of the disease presentation can be explained by reductive stress and the consequences of disrupted hepatic electron flow. Disease is exacerbated by carbohydrates and is managed with a higher protein, higher fat diet. Interestingly, CD patients remain sucrose-aversive after successful dietary management. Moreover, the sucrose-aversive effects of SLC25A13 deletion can be seen in the mouse model of CD, suggesting the existence of a liver-derived signal that may act in the ventromedial hypothalamus. Using hepatocyte cell lines with defined mutations and/or pharmacological blocks in the malate-aspartate shuttle, the glycerol-3-phosphate shuttle and the carbohydrate response element binding protein, we have dissected a transcriptional program that recapitulates human CD and which induces a neuroactive factor that we think explains the sucrose-aversive presentation of this rare disease.

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Abstract 1170

Investigating the conditionally essential role of human METAP1

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CRISPR-based forward genetic screens make it possible to systematically identify genes required for survival and growth in human cells, and therefore provide a powerful approach to characterize protein function and uncover targetable vulnerabilities in human cancer cells. Notably, it has become appreciated that gene essentiality can be context-dependent, as CRISPR-based loss-of-function screens across hundreds of human cancer cell lines have uncovered cell-essential genes that can vary with cell-intrinsic characteristics such as genotype and lineage. Importantly, environmental factors also influence cell physiology. However, there has been little consideration into how nutrient availability might impact gene essentiality, and most CRISPR-based screens of human cells are performed *in vitro* using culture media that poorly recapitulate nutrient conditions in the body. To test the hypothesis that medium composition influences gene essentiality, our lab performed genome-wide CRISPR screens of human blood cancer cell lines in traditional versus Human Plasma-Like Medium (HPLM), a physiologic medium that we previously developed to more closely reflect the metabolic composition of human blood. Among the strongest HPLM-essential hits identified from our screen results is METAP1, which encodes one of two human enzymes (METAP1 and METAP2) that can catalyze the cleavage of N-terminal methionine from nascent polypeptides. Interestingly, these two METAPs are co-expressed across most human cell lines, are each localized to the cytosol, and share seemingly similar substrate preferences. However, our screen results indicate that endogenous METAP2 cannot complement the loss of METAP1 in HPLM-cultured cells, suggesting that METAP1 serves a non-redundant and context-dependent cell-essential role. Therefore, we hypothesize that identifying the gene-nutrient interaction that underlies conditional METAP1 essentiality will provide key insights into this unforeseen role. Thus, we have engineered METAP1-knockout cells and, in turn, confirmed the conditional CRISPR phenotype for METAP1 by performing relative growth assays. We are now poised to identify the gene-nutrient interaction and will proceed to use tools in biochemistry, metabolomics, and molecular biology to explore the context-dependent role of METAP1 in proliferating human cells.

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Abstract 1197

The yeast [2Fe-2S] mitochondrial protein Aim32 supports the formation of cytochrome c oxidase complex

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Mitochondria produce energy through oxidative phosphorylation (OXPHOS), where respiratory complexes: Complex II (CII), III (CIII) & IV (CIV) oxidize substrates and transfer electrons to oxygen, generating a gradient that allows phosphorylation of ADP to ATP by the ATP synthase complex. This is a critical energy generating process occurring in the mitochondria; consequently, any disruptions within the electron transport chain (ETC) or OXPHOS impacts the overall cellular function. Studies from our laboratory previously demonstrated that Aim32, a thioredoxin-like [2Fe-2S] ferredoxin protein, is dual-localized within mitochondria and participates in redox (reduction-oxidation reactions); but the molecular mechanisms are unidentified. Yeast depleted in Aim32 activity (Δaim32) exhibited a respiratory deficiency phenotype. Several ETC proteins were identified as Aim32 binding partners in mass spectrometry analysis performed with FLAG-tagged Aim32. The relative abundance of the mitochondrial genome (mtDNA)-encoded subunits of Cytochrome c oxidase complex, CIV (Cox2, Cox3) as well as those encoded by the nuclear genome (Cox4, Cox5/6) were significantly reduced in Δaim32 mitochondria. In *S. cerevisiae*, the two proton-pumping units, CIII and CIV further assemble into Respiratory SuperComplex (RSC) structures consisting of a dimer of CIII and one or two copies of CIV [III2+IV1-2], which enhances the efficiency of electron transfer between complexes. We initially examined the organization and activity of the RSCs in mitochondrial extracts from WT and Δaim32 yeast strains, using a combination of colorless native (CN)-PAGE and blue native (BN)-PAGE. We found that CII activity was considerably reduced in mitochondrial extracts from Δaim32 yeast strain. Although reductions in CIV activity were not observed in the Δaim32 strain, free CIV monomer was clearly increased, which is suggestive of CIV remodeling. Finally, BN-PAGE analyses followed by western blot detection with antibodies against select components of the respiratory chain revealed a clear prevalence of III2IV2 over III2IV SC in WT mitochondria. In contrast, there was proportionately more of the smaller RSC (III2IV>III2IV2) in Δaim32 mitochondria and the pool of dimeric CIII was increased, whereas CV was unaffected. These alterations suggest that the steady-state level of CIV is perhaps limiting when AIM32 is nonfunctional. Aim32 may potentially be a novel regulator of cytochrome c oxidase biogenesis and consequently, affects energy transduction.

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ASBMB

Abstract 1212

Protocatechuic acid Abrogates Adriamycin-induced reproductive toxicities and hepatic damage in rats

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Adriamycin (ADR) is an anticancer drug that is well documented for its non-target toxicities in many organs including testes and liver. Hence its limitations in its use in chemotherapy. This study seeks to examine the possible effect of protocatechuic acid (PCA) in ADR-induced hepatic and reproductive toxicities in rats. Thirty male rats of average weight 170 g were divided into five groups: group 1 served as control group, group 2 was administered 20 mg/kg of ADR intraperitoneally, groups 3 and 4 were administered ADR and separate doses of PCA (10 and 20 mg/kg body weight) respectively, while the group 5 rats received only 20 mg/kg of PCA. The animals were handled and used in accordance with the National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals and also the under the guidelines of the ethical committee of the university. Pre-treatment with PCA significantly improved sperm motility, sperm count, and viability in ADR-induced rats. Furthermore, elevated levels of testes and liver malondialdehyde were significantly reduced in rats treated with PCA. Also, the increased serum alkaline phosphatase, aspartate transaminase, total cholesterol, triglyceride total protein, and albumin levels in ADR-induced rats were brought near the control value by PCA treatment. Conversely, reduction in the levels of testes and liver glutathione as well as the serum high-density lipoprotein (HDL) cholesterol level in ADR group was significantly raised in PCA-treated rats. Therefore, PCA may represent a natural and promising compound for mitigating against oxidative stress-mediated toxicities.

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Abstract 1217

Association of Dietary Intakes of Calcium/Phosphorous with Biochemical Osteomalacia and its Components

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Background: Our previous study revealed a high prevalence of abnormal mineralization markers namely low 25 hydroxyvitamin D (47.9%); high serum alkaline phosphatase (3.7%) and low calcium-phosphate product (9.8%) suggestive of biochemical signs of osteomalacia (OM, defined as any two of these risk factors). OM was more prevalent in girls (11.2%) compared to boys (5.0%). In this follow-up study, we aimed to evaluate if biochemical OM was associated with low intakes of calcium and phosphorous.

Methods: Saudi adolescents (N = 2938, 57.8% girls), aged 12–17 years from 60 different secondary and preparatory year schools in Riyadh, Saudi Arabia were included in this study. A dietary recall for daily intakes of nutrients/minerals using a validated computerized food database "ESHA—the Food Processor Nutrition Analysis program" was collected. Compliance to reference daily intake (RDI) was calculated. Fasting blood samples were collected and circulating levels of 25 hydroxyvitamin D, alkaline phosphatase, calcium, phosphate, and C-terminal telopeptide (CTX) were analyzed.

Results: A total of 1703 Saudi adolescents (991 girls, 712 boys) provided the dietary recall data. A major proportion (89.6%, 92.2%) of the participants failed to achieve the RDI of 1000 mg/day and 10 mcg/day of dietary calcium and vitamin D respectively. The average daily dietary calcium intake was significantly lower in girls compared to boys (median levels of 294.3 and 345.5 mg/day respectively, $p < 0.001$). In contrast, boys reported lower dietary intakes of phosphorous compared to girls ($p < 0.01$). Interestingly, no significant correlation in status of biochemical OM or its individual risk factors with dietary calcium intake was found, irrespective of sex. However, circulating 25 hydroxyvitamin D and alkaline phosphatase levels correlated negatively with daily dietary intakes of phosphorous in girls ($r = -0.18$, $p < 0.001$) and boys ($r = -0.14$, $p < 0.05$) respectively.

Conclusions: This study suggests a need for vitamin D fortification and increased dietary calcium in the diet of Saudi adolescents. The results also show that all adolescents exceeded the RDI for dietary phosphorous but none met the RDI for dietary calcium and vitamin D, and none had sufficient 25 hydroxyvitamin D levels (>50 nmol/L). We speculate that the high phosphate diet may somehow compensate for the insufficient supply with calcium and vitamin D. This insufficient supply would otherwise have caused a much higher prevalence of biochemical OM than the 6.2% we observed.

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Abstract 1222

The Effect of Bitter Melon (*Momordica charantia*) Extract on the Regulation of Gluconeogenesis in Liver Cells

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Jheem Medh

Background: Diabetes is widely prevalent disease. In 2022, the Center for Disease Control and Prevention reported that 11.3% of the US population (about 28.7 million people) were diagnosed with diabetes and 96 million adults had prediabetes. In Asia and Africa, the bitter melon fruit is traditionally used as a medicinal supplement for managing type 2 diabetes.

Objective: The purpose of this study was to examine the effects of bitter melon extract on the transcription of regulatory enzymes of gluconeogenesis in a human liver cell line (HepG2 cells). The phenolic content and antioxidant capacity of the bitter melon extract will also be determined.

Methods: The juice of bitter melon was squeezed from both the skin and the flesh of the fruit. The phenolic content was measured by the Folin-Ciocalteu reagent (FCR) Assay, which is a colorimetric assay using Gallic Acid as a standard. The Ferric Reducing Antioxidant Power Assay (FRAP) Assay, also a colorimetric assay, was used for detecting antioxidants at multiple capacities by the reduction of iron (III) to iron (II). HepG2 cells were incubated without or with the bitter melon extract for 2–4 days. Total RNA was isolated and specific primer pairs were used to amplify phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6PC), the regulatory enzymes of gluconeogenesis. Both endpoint reverse transcription polymerase chain reaction (RT-PCR) and quantitative polymerase chain reaction (qPCR) were used for the specific amplification, detection, and quantification of PEPCK and G6PC. β-actin was amplified as a control house-keeping gene. Results: The phenolic content of both bitter melon skin and flesh was similar at about 1 mg/mL. The FCR and FRAP assays indicated that bitter melon had high levels of phenolic content (750 to 1500 mg/L) and antioxidant capacity (20–30 g/mL). Endpoint PCR qualitatively showed that the transcription of PEPCK and G6PC were reduced in HepG2 cells treated with bitter melon extract, compared to controls. Analysis of Ct values obtained by qPCR by the ΔΔ Ct method showed that PEPCK and G6PC transcript levels were reduced by >80% in cells treated with bitter melon extract. Conclusion: Our study indicates that bitter melon may be beneficial to diabetics by reducing their capacity for gluconeogenesis and thereby facilitating the management of blood glucose levels.

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ASBMB

Abstract 1266

Arginyltransferase co-evolves with mitochondria and mediates oxygen sensing in different eukaryotic kingdoms

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The response to oxygen availability is a fundamental process concerning metabolism and survival/death in most organisms. In eukaryotes, the needs for oxygen sensing must have emerged along with the acquisition of mitochondria. The eukaryotes share one common ancestor of mitochondria, which arose only one time in the evolution. As such, it is expected all eukaryotes should share at least one common molecular pathway for oxygen sensing. However, this hypothesis was not supported by high diversity among the known oxygen sensing mechanisms in different eukaryotic kingdoms. In the Plantae kingdom, oxygen sensing is known to be mediated by Arginyltransferase (ATE), which catalyzes a posttranslational modification called arginylation to induce protein degradation. Here, we present evidence showing that oxygen sensing in the Animalia and fungi kingdoms are also mediated by the ATE enzyme. Particularly, in animals, ATE centrally controls hypoxic response and glycolysis by modifying HIF1a in a manner that is dependent on the availability of oxygen. Furthermore, we showed that the ATE-dependent pathway is separate from the action of pVHL E3 ubiquitin ligase, the conventional oxygen sensing pathway discovered in animals. Bioinformatic and phylogenetic analysis suggests that eukaryotic ATE1 likely evolved during mitochondrial domestication as a baseline mechanism for oxygen sensing, while additional pathways were acquired later on to accommodate differential needs for oxygen sensing in different organisms. Therefore, our research suggest that the ATE-mediate mechanism is a common, ancestral oxygen sensing pathway among different eukaryotic kingdoms.

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Abstract 1292

Reactive Oxygen Species Production and Regulation in Human Cell

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The seven NADPH oxidoreductase (Nox) isoform (Nox1-5, Duox1 and Duox2) expressed in human tissue can be distinguished by their tissue distribution, structure and reactive oxygen species (ROS) product. All seven Nox isoform proteins consist of highly conserved structural features as flavocytochrome; the C-terminal cytosolic dehydrogenase (DH) domain consists of FAD and NADPH binding sites. In addition, their N-terminal transmembrane (TM) region consists of a six α-helical structure that involves four conserved histidine residues, located in the third and fifth TM helices that co-ordinate two heme groups (heme bL and bH). Non-phagocytic Nox1, Nox3 and phagocytic Nox2 form heterodimers with gp91phox and p22phox in the membranes, and also required additional cytosolic regulatory proteins for their catalytic function. Constitutively active Nox4 associated with p22phox in the membranes does not require cytosolic proteins, indicating that Nox4-dependent ROS generation is regulated primarily by its expression level. Nox5 and the dual oxidase isoform (Duox1 and 2) are activated by Ca2+ binding with their N-terminal cytosolic EF-hand motif. Besides Nox and Duox family-dependent ROS production in human cells, superoxide generation is due to electron-leak in electron transport system of mitochondria and also from cytochrome P-450 in endoplasmic reticulum. Thus, human cell produces not only ROS, but also induce and activate anti-oxidative enzyme system to keep defensive function of normal cell. In many cancer cells persistently up-regulated ROS-dependent signaling pathways are involved in cell differentiation, growth and survival. Inhibition of cancer growth is required by a novel ROS-modulating agent with ability to eliminate the growth and survival of tumor cells. Inhibition of cancer growth is required by a novel ROS-modulating agent with ability to eliminate the growth and survival of tumor cells.

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Abstract 1306

Investigating the Conditionally Essential Role of BCL2L1 in Human Cells

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Jason Cantor

CRISPR-based forward genetic screens have been used to identify genes essential for cell growth and how these genes can vary by cell genotype and lineage. However, most such screens have been carried out in traditional cell culture media that poorly recapitulate the biochemical conditions cells are exposed to in the human body. To investigate how medium composition affects gene essentiality, our lab performed genome-wide CRISPR-based screens of the chronic myelogenous leukemia cell line K562 in traditional (RPMI 1640) versus Human Plasma-Like Medium (HPLM). HPLM is a physiologic cell culture medium we previously developed that contains over seventy metabolites at concentrations representative of average adult human plasma. We identified hundreds of conditionally essential genes that collectively span many different biological processes. Among the strongest scoring RPMI-essential hits was BCL2L1. This gene encodes Bcl-xL, an anti-apoptotic member of the large and well-studied B-cell lymphoma 2 (BCL2) family. Interestingly, BCL2L1 was the only family member identified as conditionally essential in our screens, suggesting it has a non-redundant, context dependent role. Therefore, we reason that identifying the gene-nutrient interaction that underlies conditional BCL2L1 essentiality will provide key insights to help uncover its role. To begin to investigate this, we have engineered BCL2L1-knockout K562 cells and confirmed the phenotype suggested by our screen using relative growth assays. While the gene-nutrient interaction is not immediately apparent, our preliminary data suggest that it involves one or more of the thirty components uniquely defined in HPLM and absent from the defined formulation of RPMI. In future work, we will identify the gene-nutrient interaction and use tools in biochemistry, metabolomics, and molecular biology to arrive at a mechanistic understanding of the conditionally essential role of BCL2L1.

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Abstract 1345

Metabolic regulation allows macrophages to maximize host defense and minimize tissue damage

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Macrophages are activated during microbial infection to coordinate inflammatory responses and host defense. However, how such inflammatory responses are regulated commensurate with the degree of microbial infection is not well understood. Here we show that in macrophages activated by bacterial lipopolysaccharide (LPS), mitochondrial glycerol 3-phosphate dehydrogenase (GPD2) regulates glucose oxidation to drive inflammatory responses. GPD2, a component of the glycerol phosphate shuttle (GPS), boosts glucose oxidation to fuel Acetyl-CoA production, histone acetylation and inflammatory gene induction. While acute LPS exposure drives macrophage activation, prolonged exposure triggers entry into LPS tolerance, in which macrophages orchestrate immunosuppression to limit the detrimental effects of sustained inflammation. We find that the shift in the inflammatory response is modulated by GPD2, which coordinates a shutdown of oxidative metabolism that limits Acetyl-CoA availability for histone acetylation at inflammatory genes, thus contributing to suppression of inflammatory responses. Therefore, GPD2 and the GPS integrate the extent of microbial stimulation with glucose oxidation to balance the beneficial and detrimental effects of the inflammatory response, maximizing host defense while minimizing tissue damage.

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ASBMB

Abstract 1374

Association of genetic polymorphism of xenobiotic metabolism enzymes and oxidative stress with prostate cancer risk in Bangladeshi population

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Yearul Kabir, Tahminur Rahman, SK Farhad Munir

One of the leading causes of cancer-related mortality in male is prostate cancer (PCa). The etiology of prostate cancer is not well understood; however, studies examining genetics, diet, lifestyle, and certain chemicals exposure are increasingly attracting attention. Polymorphic distribution of the xenobiotic enzymes involved in the activation and/ or deactivation of aromatic amines in humans is an important determinant of individual susceptibility to their carcinogenic effects. Prostate epithelial cell express phage II metabolizing enzymes and recent molecular epidemiological studies have analyzed the relationship between xenobiotic enzymes such as, N acetyl transferase (NAT) and Glutathione S transferase (GST) gene polymorphism in etiology of prostate cancer. Oxidative stress has long been implicated in cancer development and progression. The expression of several enzymes involved in oxidative stress and detoxification is repressed in prostate cancer, in particular, glutathione S-transferase. Therefore, this study is aimed to investigate the association of genetic polymorphism of xenobiotic enzymes and the influence of oxidative stress in prostate cancer patients in the Bangladeshi population. This case-control study included 207 histopathologically diagnosed cases of prostate cancer and 200 age-matched healthy controls. After taking informed written consent, preset questioners were filled up, and about 06 ml of venous blood were collected with all ascetic precaution from each study subject. 03 ml blood was collected in EDTA vial to extract genomic DNA for genetic analysis of NAT2, GSTT1 & GSTM1 by PCR-RFLP by multiplex PCR methods. Remaining 03 ml blood were used for estimation of serum PSA, and oxidative stress markers including malondialdehyde (MDA), Glutathione S transferase (GST), Superoxide dismutase (SOD) and erythrocyte reduced glutathione (GSH). All data were plotted in SPSS version 23, and different statistical analyses were done. In this study, the mean ± SD age of cases and control was 67.3 ± 8.3, and 62.2 ± 6.8 years, respectively. A higher frequency of mutant NAT2*5A, NAT2*6A and NAT2*7A in prostate cancer cases were observed in this study. Prostate cancer risk was found considerably increased in patients with NAT2 slow genotypes, GSTT1 and GSTM1 null genotypes, compared to control. Furthermore, Prostate cancer risk was found very significantly associated with the presence of combined genotypes that included NAT2 (slow), GSTT1 (null), and GSTM1 (null), and the risk rose 9.64 fold when compared to the normal genotypes. Again, it was observed that, individual with positive smoking history/family history of cancer along with NAT2 slow genotype had significantly increased risk for prostate cancer. Moreover, the likelihood of developing a moderate to high-grade tumor was found considerably greater in persons with NAT2 slow genotypes, GSTT1 and GSTM1 null genotypes. Oxidative stress marker MDA was found significantly increased, antioxidant GSH and SOD were significantly reduced, and GST activity was significantly increased in prostate cancer patients, compared to control group. In conclusion, this study established the association of genetic polymorphisms of NAT2, GSTT1, and GSTM1 gene and oxidative stress with prostate cancer risk in Bangladeshi population. So, identification of susceptible individuals and screening of oxidative stress and antioxidant status in the elderly male is recommended to develop preventive strategies and proper management of prostate cancer.

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Abstract 1415

Modulatory effect of *Azadirachta indica* leaf extract in mitigating isoproterenol induced cardiotoxicity

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Cardiotoxicity involves damage to the heart's cells with age-related conditions being a predominant factor. Cardiotoxicity may arise because of cellular damage due to the formation of free oxygen radicals, oxidative stress, and the induction of immunogenic reactions with the presence of antigen presenting cells in the heart. The present study investigated the antioxidative and cardioprotective effect of *Azadirachta indica* (AI) on Isoproterenol induced cardiotoxicity together with possible molecular mechanism of action. The animals were randomly divided into five (5) experimental groups with ten (10) animals in each group, and the treatment was as follows: Corn oil was administered orally for 14 days to Group A. 100 mg/kg Isoproterenol (ISO) was administered intraperitoneally on days 15 & 16 respectively to Group B. Groups C and D were treated with *A. indica* (AI) at 100 and 200 mg/kg for 14 days followed by ISO at 100 mg/kg intraperitoneally on days 15 & 16 respectively. Group E received Clofibrate orally at 300 mg/kg for 14 days and ISO intraperitoneally on days 15 & 16 respectively. Blood pressure parameters, markers of oxidative stress and cardiac damage were measured. The immunohistochemistry of cardiac injury was also determined. Results showed significant elevation in the cardiac and renal oxidative stress markers and reduction in antioxidant status in ISO intoxicated rats. Immunohistochemistry revealed reduced expressions of PPARα and BCL2 in the cardiac and renal tissues of ISO-only treated rats. However, pre-treatment with *A. indica* mitigated both cardiac and renal oxidative stress. Reduced serum NO level and blood pressure were normalized in rats pre-treated with *A. indica* with concomitant higher expressions of PPARα and BCL2 in the cardiac and renal tissues. Findings from this study suggest that pre-treatment with AI (100 and 200 mg/kg) offered antioxidative and cardioprotective effect through reduction of oxidative stress and up-regulation of PPARα and BCL2 signaling.

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Abstract 1503

Extracellular electron transfer in Shewanella and other microbes

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Iron is a key element for most life on earth and can serve as an acceptor or donor for electrons in dissimilatory iron reducing bacteria or neutrophilic iron oxidizing bacteria, respectively. Iron is primarily found in an oxidized, insoluble state such that iron reducers must deliver electrons to a substrate located extracellularly. For iron oxidizing bacteria living at near neutral conditions, iron oxide is the byproduct of their metabolism meaning that electrons must be taken into the cell from the exterior. I will discuss the latest mechanisms for electron transfer in both iron reducing and iron oxidizing bacteria and how we can gain mechanistic insight into these processes by rebuilding pathways in engineered chassis strains. The rate of extracellular electron transfer varies across related strains (Shewanella, Aeromonas and Vibrio) despite using the same strategy to deliver electrons across the outer membrane. Iron oxidizing bacteria are challenging to cultivate and manipulate in laboratory conditions. Using engineered chassis strains that lack extracellular electron transfer components, we have reconstructed components from putative iron oxidation pathways, providing insight into their functionality. Studying extracellular electron transfer and uptake systems not only illuminates the unusual metabolic capabilities of these microbes but also facilitates their use in electrochemical systems for applications in bioenergy and biocatalysis.

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ASBMB

Abstract 1507

Characterization of a novel pre-clinical model of alcohol-associated hepatitis: association between compromised liver regeneration and cardiolipin synthesis

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Background: Alcohol-associated hepatitis (AH) is a severe clinical manifestation of alcohol-associated liver disease (ALD) with high mortality due to chronic liver injury, compromised mitochondrial function, and inadequate liver regeneration. Cardiolipin is a functional constituent of the mitochondrial membrane that maintains mitochondrial energy production to meet the metabolic demand of hepatocyte proliferation during regeneration. In this study, we aimed to develop a novel experimental model of AH that more closely mimics features of human AH including liver injury, endotoxemia, and impaired liver function and regeneration. Further, we aimed to examine the association between liver regeneration and hepatic cardiolipin metabolism in this model.

Methods: Male WT C57Bl/6J mice were fed ethanol (EtOH) or a control pair-fed (PF) diet for 10 days ± galactosamine (GalN) at 500 mg/kg i.p. for 3 days prior to sacrifice (n = 10 per group). EtOH-fed mice received a one-time EtOH binge (5 g/kg) on day 11. GalN was used due to its ability to induce robust liver injury by sensitizing the liver to gut-derived endotoxin. Liver injury endpoints including plasma ALT and hepatocyte necrosis were evaluated. Plasma LPS and albumin were measured to assess endotoxemia and liver function, respectively. Liver regeneration was evaluated by EdU and PCNA staining. Hepatic cardiolipin levels and gene expression of enzymes involved in cardiolipin metabolism were measured by fluorometric assay and qPCR, respectively. One-way ANOVA was used to determine statistical significance ($p < 0.05$).

Results: Plasma ALT levels were elevated in PF-GalN and EtOH-GalN mice relative to PF and EtOH controls. Hepatocyte necrosis and endotoxemia were increased in EtOH-GalN as compared to PF-GalN mice. Liver function was impaired in EtOH-GalN vs PF-GalN mice as determined by reduced plasma albumin levels. In addition, liver regeneration was compromised in EtOH+GalN mice as determined by reduced EdU+ and PCNA+ hepatocytes and Afp gene expression, which was not observed in PF-GalN mice. Further, hepatic cardiolipin levels were reduced in EtOH+GalN mice relative to PF+GalN mice. Finally, expression of genes involved in cardiolipin synthesis such as Pgs1, Ptpmt1, Crls1, and Taz was significantly lower in EtOH+GalN compared to PF+GalN mice.

Conclusion: In this study we developed a new experimental model that recapitulates features of human AH including liver injury, endotoxemia, and impaired liver function and regeneration. We also demonstrated that liver regeneration and cardiolipin synthesis were both compromised in this pre-clinical model. Further studies to determine if treatments tailored to increase hepatic cardiolipin would enhance liver regeneration are warranted.

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Abstract 1542

ATP10A has a protective and sex-specific role in lipid metabolism

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Genetic predisposition and environment play substantial roles in obesity, type 2 diabetes and cardiovascular disease (CVD). Genetic association studies have linked ATP10A, encoding a type IV P-type ATPase (P4-ATPase), to human metabolic disease. ATP10A is a lipid flippase that catalyzes the membrane translocation of phosphatidylcholine and glucosylceramide. These lipids and their respective metabolites have been independently implicated in metabolic dysfunction. For these reasons, we wanted to determine ATP10A's role in metabolism. To explore the role of this flippase in metabolism, we created a novel Atp10A knockout (KO) mouse model. We found that Atp10A KO mice display a female-specific weight gain during high-fat diet feeding and this is attributable to increased adiposity. Female Atp10A KO mice also exhibit elevated plasma free fatty acids, cholesterol, and triglycerides, as well as a depletion in eicosanoid species compared to the wild type (WT) littermates. Additionally, female Atp10A KO mice exhibit elevated fasting blood glucose levels without compensatory elevation of insulin. We also found that the liver of female Atp10A KO mice displays larger lipid droplets, which was associated with increased diacylglycerol acyltransferase-2 (DGAT2) expression and an attenuation of the insulin signaling pathway compared to the WT littermates. Thus far, our studies have shown that knocking out Atp10A in mice on a high fat diet results in sex-specific perturbations to body composition, plasma lipid levels, glucose homeostasis, and metabolic signaling in the liver. Interestingly, we discovered that ATP10A is expressed in vascular endothelial cells of multiple tissues and that Atp10A knockdown in this cell type alters eNOS activation. We are now exploring the previously described mouse metabolic phenotypes through the lens of vascular endothelial cell dysfunction. These studies suggest mechanisms by which this flippase contributes to the development of CVD with obesity, perhaps by effecting vascular health.

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Abstract 1601

Defying hypoxia: using weakly electric fishes to better understand the molecular basis of hypoxia tolerance

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Oxygen deprivation is a major metabolic stress that causes cell death and underlies many human pathologies, like cancers. Despite the detrimental effects of hypoxia, many examples of aquatic organisms display exceptional ability to survive lethal levels of hypoxia. Species that live in habitats that experience seasonal levels of hypoxia and anoxia have been shown to be far more tolerant than other animals. In this study, we use *in-vitro* assays to investigate the molecular mechanisms of hypoxia tolerance using a group of weakly electric fishes that evolved hypoxia tolerance multiple times. We show that hypoxia tolerance in these fishes likely evolved via differences in transcriptional regulation, as well as coding sequence evolution of genes involved in the hypoxia response. We identify motifs in the master regulator of hypoxia response, hypoxia inducible factor 1α, that facilitate its enhanced function in tolerant species. Taken together, our study enriches our understanding of molecular mechanisms of hypoxia tolerance and suggests novel regulatory mechanisms of hypoxia inducible factor 1α.

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Abstract 1618

Exploring the effect of prepregnancy obesity and circadian disruption on milk production efficiency in mice

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Breast milk is the ideal food for the growing neonate, however, only 30% of infants receive the recommended amount of breastmilk for healthy long-term development. There are two maternal conditions that we believe heavily contribute to this low percentage: prepregnancy obesity and circadian disruption, which is caused by disrupted sleep. Prepregnancy obesity is associated with early cessation of breastfeeding, and circadian disruption was found to be related to early supplementation with milk formula. We hypothesized that prepregnancy obesity and circadian disruption were negatively impacting the ability of mothers to produce milk. The amount of milk produced by the mammary gland is determined by the number of cells and the metabolic activity of those cells. Mitochondria produce 90% of the energy used to supposed metabolic processes, and thus, the number of mitochondria in the tissues of a mother may affect her milk production levels. The objective of this study was to determine the effect of prepregnancy obesity and exposure to continuous light, which disrupts the circadian rhythm, on the relative number of mitochondria, activity of the mitochondrial enzyme succinate dehydrogenase (SDH), and ATP concentration in mouse mammary glands at peak lactation. A 2X3 factorial-designed study of mice fed a high-fat (HF) or control (CON) diet and exposed to 12 hours of light and 12 hours of dark (LD), continuous light at 5 lux (L5), or continuous light at 100 lux (L100) was conducted to determine the effect of treatments on litter growth during lactation, number of mitochondria, SDH activity, and ATP concentration in the mammary gland. DNA extraction and qPCR were used to determine the mitochondrial to chromosomal DNA ratio, and thus, the ATP concentration. Diet had an overall effect on litter growth, with litters of HF dams weighing significantly more than CON. Light had a tendency ($P = 0.06$) to affect litter growth, with weights of L5 and L100 litters greater than LD. Neither diet nor light affected number of mitochondria ($P > 0.05$). SDH staining was not affected by diet nor light, but ATP content of mammary was significantly ($P < 0.001$) affected by light with L5 and L100 having lower ATP than LD dams. Analysis is ongoing, but if ATP reflects metabolic output, these findings suggest that exposure to continuous light may increase milk production efficiency in mice.

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Abstract 1655

Investigating the unexpected effect of phosphorylation on isocitrate dehydrogenase 2 activity

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Best known as "the powerhouses of the cell," mitochondria are central hubs for metabolic processes. While numerous reactions catalyzing nutrient catabolism occur within mitochondria, the mechanisms underlying their regulation are not fully known. Phosphorylation, as a rapid and reversible modification, is well positioned to regulate these metabolic reactions, yet our understanding of phosphorylation-mediated regulation on mitochondrial proteins is lacking. Our lab found that knockout of mitochondrial protein phosphatase Pptc7 in mice leads to the hyperphosphorylation of specific mitochondrial proteins, resulting in metabolic defects such as hypoketotic hypoglycemia. When Pptc7 is knocked out across multiple models (i.e., mice and cells), isocitrate dehydrogenase 2 (Idh2; human homolog IDH2) is reproducibly hyperphosphorylated at serine residue 423. IDH2 contributes to tricarboxylic acid (TCA) cycle flux, oxidative stress protection, and biosynthetic processes. Notably, IDH2 is mutated in multiple cancers, but the post-translational regulation of IDH2 is not yet well understood. To decipher the functional consequences of IDH2 phosphorylation, we have generated IDH2 mutants that mimic constitutive phosphorylation (i.e., phosphomimetic mutations) and conducted lysate-based enzyme assays. These assays demonstrate that phosphomimetic mutation of serine 423 significantly elevates IDH2 enzyme activity, unexpectedly contradicting the role of phosphorylation on *Escherichia coli* homolog IDH. These results suggest that phosphorylation regulates IDH2 activity, potentially contributing to the regulation of multiple critical metabolic processes.

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Abstract 1721

Glycerate production from intestinal fructose metabolism is increased by dietary fat, which contributes to islet cell damage and glucose intolerance

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Type II diabetes mellitus (T2DM) has doubled in prevalence over the last two decades, becoming a major threat to global health. Although T2DM is associated with genetics, the development of glucose intolerance is also a consequence of lifestyle and dietary patterns. Ample evidence associates the consumption of fructose-containing sweetened beverages, as part of the western diet, with the development of T2DM. Dietary fat, a prominent component of the western diet, has also been implicated in obesity and T2DM. Studies that analyzed the combined effects of fat and mono-saccharide consumption found that fructose, but not glucose, exaggerates the deleterious effects of dietary fat. Reciprocally, pathogenic effects of fructose are more prominent among mice fed a highfat diet, although the mechanisms remain unknown. High fructose feeding is a well-established model for inducing insulin resistance. Fructose ingestion does not impact insulin-secreting β-cells directly, as low expression of fructose transporters impedes their metabolism of fructose, but synergizes with glucose in signaling to β-cells. Although hyperinsulinemia could result from increased calorie intake and adiposity upon high fructose consumption, fructose metabolism in the liver seems to be the main contributor to metabolic dysfunction. Compared with glucose catabolism, unchecked fructose catabolism can initiate rapidly and produce a flood of acetyl-CoA and glycerol-3-phosphate that can fuel de novo synthesis of triglycerides and the development of steatosis. Metabolites from both dietary fats and carbohydrates can regulate each other through master transcriptional regulators like carbohydrate response element-binding protein (Chrebp), peroxisome proliferator activated receptor gamma (Pparg), and sterol regulatory element-binding protein-1c (Srebp-1c or Srebf)). Hepatic fructose metabolism can also deplete local adenosine triphosphate (ATP) and generate significant amounts of uric acid, although their association with clinical hepatic insulin resistance and steatosis is still under debate. Recent reports demonstrated that the intestine largely shields the liver and other internal organs from the effects of fructose consumption, such as enhanced lipogenesis and ectopic fat deposition, by converting a substantial amount of ingested fructose to various metabolites. Only when ingested fructose exceeds the maximum fructose metabolism capacity of the small intestine does unconverted fructose "spillover" to the liver and to the colon. These findings indicate that the metabolism of fructose is not localized to the liver and that a systematic evaluation of its fate is warranted. However, little is known about the contribution of fructose-derived metabolites from the small intestine to the development of T2DM, particularly in a liver-independent fashion. We observed that a high-fat diet (HFD) altered fructose metabolism in the mouse small intestine, resulting in an enrichment of organic acids from the fructolysis pathway but not the citric acid cycle. In particular, fructose-derived glycerate was sustained at high levels in systemic circulation, and chronic glycerate treatment caused long-term β-cell damage *in vivo*. These data suggest that fat consumption enhances intestinal fructose metabolism and circulation of its derived glycerate, which leads to chronic damage to pancreatic β-cells and subsequent glucose intolerance. Our model provides a mechanism linking T2DM pathogenesis to consumption of a western diet rich in fructose and fat.

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ASBMB

Abstract 1767

Isolation and Proteomics of the Insulin Secretory Granule

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AIMS: Pancreatic β-cells store insulin in vesicles terms insulin secretory granules (ISGs). Following glucose stimuli, ISGs are triggered for exocytosis, releasing insulin. Only 1–5% of total ISGs are targeted for secretion, and it is not known how beta-cells select ISGs for secretion. Plasma membrane proximity and docking are mechanisms classically described to dictate ISG secretion but recently, ISG age and motility have also been shown to contribute to ISG selection for secretion. Many processes control ISG turnover, including biogenesis, trafficking, and degradation, which may be regulated externally but also by proteins both in the lumen and on the membrane of ISGs. Previously, only 4 studies published show ISG proteins within rat insulinoma β-cells (INS1 or INS1-E), with 5 proteins in consensus. The aim of this project was to further elucidate proteins both on and in the ISG within the mouse insulinoma β-cell line (MIN6) that may dictate or control ISG selection for secretion. Methods: To identify potential ISG proteins, MIN6 mouse insulinoma β-cells were subjected to a three-step purification method to enrich for ISGs. These included two subcellular fractionations with Optiprep, Percoll and a third sucrose enrichment of ISGs. Western blot analysis validates enrichment and purification of ISG with the exclusion of contaminating proteins. ISG enriched fractions were then analysed by LC-MS/MS. Following this, protein correlation profiling was used to obtain a proteome of MIN6 ISGs. Further analyses were performed to obtain protein profiles of all proteins to assigned subcellular locations using LOPIT-DC. A total of 8000 proteins were identified across fractions, and with downstream analyses, 500 total candidate proteins were identified to be ISG proteins.

CONCLUSIONS: Together, these data provide an efficient and transferrable method of isolating ISGs from β-cells and candidate proteins for further validation in ISGs.

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Abstract 1787

A novel link between mitochondrial energy metabolism and ubiquitin conjugase Rad6

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Oxidative stress, where cells accumulate reactive oxygen species (ROS), is a harmful phenomenon known to damage biomolecules, foster cell death, and contribute to aging and neurodegeneration. In response to stress, our group found that the multifunctional ubiquitin conjugase Rad6 regulates protein translation via ribosome ubiquitination. Additionally, we found that the deletion of RAD6 (*rad6Δ*) in yeast results in reduced cellular growth, ROS-accumulation, and an increased expression of mitochondrial genes. In this work, we illustrate that in the absence of Rad6, compensatory mechanisms at the mitochondria are employed to sustain yeast cell viability. We first showed that *rad6Δ* has greater levels of the mitochondrial protein porin than the WT strain, suggesting that the mitochondria biology in *rad6Δ* may be altered. Yeast cells primarily undergo fermentation as their main energy source, which does not require mitochondria, suggesting that absence of Rad6 leads to a reprogramming of energy metabolism. Through fluorescence microscopy, we showed that the deletion of RAD6 increases the amount of active and ROS-producing mitochondria, supporting the idea that *rad6Δ* may be performing cellular respiration rather than fermentation. To test this hypothesis, we incubated the WT and *rad6Δ* strains with mitochondrial-inhibiting drugs, CCCP and antimycin, and found that *rad6Δ* growth was more susceptible to both. To investigate whether increased ROS accumulation results in more dysfunctional mitochondria in the *rad6Δ* strain, we sought to measure the selective degradation of mitochondria through mitophagy. Using a fluorescent mitophagy reporter, we observed higher levels of mitochondrial protein degradation in the *rad6Δ* strain in comparison to the WT. Corroborating our model, analysis of gene expression profiles further indicates that cells lacking Rad6 switch to cellular respiration for energy production. Because Rad6 is involved in controlling gene transcription and translation, further investigation of Rad6's role in yeast energy metabolism will yield novel insights into fundamental processes of gene expression and cellular adaptation to dynamic environments.

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Abstract 1833

Glutathione signaling ensures the preservation of stem cell self-renewal capacity in a nutrient-depleted environment

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Adult muscle stem cells reside within a niche which controls self-renewal and differentiation decisions. While slow cycling rates and fatty acid-derived oxidative phosphorylation are hallmarks of quiescent muscle stem cells, activated muscle stem cells experience a rapid burst of proliferation required for myonuclear fusion. Recent works, including from our lab, have demonstrated the roles mitochondria-derived metabolites play in cell fate decision during the transition from quiescence to activation. For example, mitochondrial glutamine metabolism stimulates acetylation and nuclear translocation stem cell specific protein kinase, PASK. Nuclear PASK interacts and phosphorylates its substrate, WDR5 to drive the exit from self-renewal. Thus, mitochondrial glutamine metabolism plays a key role in adult muscle stem cells to generate the differentiation competent progenitor population. To further expand our understanding of role of mitochondrial glutamine metabolism in regulating cell-fate decisions, we investigated the functional requirement of SLC25 family of mitochondrial solute carriers during adult muscle stem cell activation, proliferation, and differentiation in the presence or absence of glutamine supplemented in the media. We identified Slc25a39 as a glutamine depletion induced transcript in proliferating myoblasts. Slc25a39 is a mitochondrial transport protein that has been found to be necessary for the transport of glutathione (GSH), a predominant quencher of reactive oxygen species (ROS), into the mitochondria. Using qRT-PCR, immunofluorescent imaging, and Western blot analysis, we show that genetic loss of Slc25a39 abrogated the exit from self-renewal, resulting in the inhibition of the myogenesis program. Building on these observations, using multi-omics analysis of stem cells in various stages of activation, proliferation, and differentiation, we have uncovered a novel mitochondria-nucleus signaling pathway anchored by glutathione that establishes a precise redox balance needed to exit from self-renewal. Thus, our results identified a novel role of mitochondrial glutamine metabolism in linking cellular redox state with cell fate decision.

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Abstract 1844

Sulindac enhances the effect of doxorubicin on pancreatic cancer cells

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers and the fourth leading cause of cancer-related deaths. The five-year survival rate has not been significantly improved using current treatment methods, including chemotherapy, immunotherapy, and surgery. Our previous findings demonstrated that sulindac, an FDA-approved non-steroidal, anti-inflammatory drug (NSAID), can sensitize cancer cells to agents that cause oxidative damage. In contrast, we have also shown that sulindac protects normal retinal, cardiac, and neuronal cells from reactive oxygen species (ROS) induced oxidative damage. Doxorubicin is an effective anticancer drug, but its use is limited due to its cardiotoxicity, which is thought to involve oxidative damage. We hypothesize that a dual drug treatment, using sulindac and doxorubicin, should enhance the efficacy of doxorubicin in killing cancer cells while protecting the heart against cardiotoxicity. For these studies, BxPC-3 and HPAF-II PDAC cell lines were used. We optimized the individual dose concentration for both sulindac and doxorubicin, as well as the drug-to-cell ratio, to obtain the best synergistic killing effect. Cell viability was measured using an MTS assay. Our results demonstrate that sulindac enhances the killing effect of doxorubicin in PDAC cells. A significant increase in cell death was observed in PDAC cells when doxorubicin was used in combination with sulindac than when each of the drugs was used alone. Preliminary data suggest that the ratio of cell density to drug amount is a critical factor in optimizing the synergistic effect. These data indicate that combining sulindac and doxorubicin, two FDA-approved drugs, may provide a new therapeutic approach to treating pancreatic and other cancers.

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Abstract 1849

MEMO1: a molecular link between iron, copper, and cancer metastasis

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MEMO1 is an evolutionary conserved protein implicated in many biological processes, however its primary molecular function remains unknown. Importantly, MEMO1 is over-expressed in many types of cancer, in particular, in breast cancer and lung cancer, and modulates cancer metastasis through altered cell motility. MEMO1 has been reported to be a copper-dependent redox-active protein, but its metal-binding properties have not been fully investigated. To understand the functional role of MEMO1, we have analyzed its genetic interactions using DepMap gene essentiality data and found multiple iron- and copper- related genes exhibiting genetic relationships with MEMO1. These interactions indicated that cells with high MEMO1 expression levels are hypersensitive to the disruptions in iron distribution. We experimentally confirmed several interactions between MEMO1 and iron-related proteins, including transferrin and transferrin receptor 2 (TFR2), aconitase/iron response protein (ACO1) and mitoferrin-2 (SLC25A28). Our inhibitor studies indicate that MEMO1 is involved in ferroptosis and is linked to iron supply to mitochondria. We have shown that purified MEMO1 binds iron or copper under redox conditions mimicking intracellular environment and solved MEMO1 structures in complex with these metals. We found that metal coordination mode in MEMO1 is very similar to that of the iron-containing extradiol dioxygenases, which also display a similar structural fold. Taken together, our results indicate that MEMO1 binds iron and regulates iron homeostasis in the cell. The ability of MEMO1 to bind either iron or copper *in vitro* suggests an intriguing possibility of a functional switch triggered by copper binding in a specific local environment within the cell. Our analysis of MEMO1 genetic interactions identified several potential drug targets for chemotherapy of high-MEMO1 cancers exploiting the synthetic dosage lethality principle.

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Abstract 1876

Differential Expression of Mitoregulin Could Decrease Long-Chain Fatty Acid Beta-Oxidation in Pancreatic Beta Cells

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Micropeptide dysregulation has been associated with multiple diseases, but this has been understudied in type 2 diabetes. A long non-coding RNA called LINC00116 codes for a micropeptide called Mitoregulin (Mtln) which enhances beta-oxidation of long-chain fatty acids. If Mtln is naturally expressed in pancreatic beta-cells, could its dysregulation cause long-chain fatty acid buildup, hinder glucose-stimulated insulin secretion, and lead to elevated blood sugar levels? For the purposes of this study, INS-1 (rat insulinoma) cells were used as a model for human pancreatic beta-cells. We performed reverse transcriptase quantitative PCR (qRT-PCR) using RNA from INS-1 cells to demonstrate, for the first time, the expression of endogenous LINC00116 in this cell line. We performed MTS assays to show that Mtln overexpression does not significantly alter fatty acid beta-oxidation in INS-1 cells. Knockdown experiments were conducted using small interfering RNA to study how Mtln knockdown affected fatty acid beta-oxidation. We performed Western blotting with an Anti-Mtln primary antibody to confirm that Mtln is endogenously expressed in INS-1 cells. These findings indicate that Mtln is naturally expressed in INS-1 cells and could have a role in pancreatic beta-cell function. This is important because Mtln has never been studied in pancreatic beta-cells and it may be a key regulator in glucose-stimulated insulin secretion.

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Abstract 1889

Effects of GLUT1 silencing on doxorubicin-mediated growth suppression and the PI3K/Akt pathway in a triple-negative breast cancer cell line

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One of the causes of chemoresistance is altered metabolic processes which can lead to tumorigenesis. Overexpression of Glucose transporter 1 (GLUT1) in cancer cells favors metastasis and drug resistance. The objective of the present study was to examine the role of GLUT1 on the PI3K/Akt pathway and its role in enhancing growth reduction by doxorubicin in triple-negative breast cancer cell line, MDA-MB-231. Using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) cell proliferation assay, we observed that suppression of GLUT1 with the chemical inhibitor WZB117 reduced the growth of MDA-MB-231 in a dose-dependent manner and improved the efficacy of doxorubicin-mediated growth suppression. Corroborating these findings, silencing GLUT1 with siRNA enhanced the cytotoxic effects of doxorubicin. Furthermore, transfection of MDA-MB-2321 cells with GLUT1 siRNA reduced the activation of the PI3K/Akt as observed through western blot analysis. These findings indicated that GLUT1 is involved in reducing the efficacy of doxorubicin by suppressing its antiproliferative functions and may mechanistically involve the activation of the PI3K/Akt pathway. To target GLUT1, we treated MDA-MB-231 cells with the polyphenol curcumin and observed a reduction in GLUT1 protein expression, suggesting the use of curcumin to alter cancer metabolism and improve drug resistance. We propose that these studies will contribute to the understanding of how GLUT1 is involved in reducing the activity of antitumor drugs in triple-negative breast cancer, and encourage the use of a nontoxic natural product like curcumin to target cancer metabolism and improve response to cancer therapeutics.

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Abstract 1893

Transcriptomic analysis of apically presented γ-glutamyl valine and glutamyl valine in murine enteroids

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Kaustav Majumder, Regis Moreau

Certain legumes and fermented foods contain γ-glutamyl peptides that interact with the calcium sensing receptor (CaSR) in the oral cavity to produce the sought after Kokumi flavor. In addition to the role of flavor enhancers, γ-glutamyl peptides have biological properties that may be useful in the prevention and treatment of chronic metabolic disorders such as type 2 diabetes, hypertension, and metabolic syndrome. Previous studies from our groups showed that γ-glutamyl valine (γ-EV) lowered blood glucose in obese diabetic db/db mice. However, intestine and liver CaSR signaling did not mediate these metabolic effects suggesting an alternative mode of action. In the present study, we investigated the signaling and mechanism of action γ-EV and EV in the gastrointestinal tract using murine 3D intestinal organoids. To that end, jejunal crypts were isolated from C57BL/6J mice and differentiated into enteroids with the brush border membrane facing outward before exposure to γ-EV or EV followed by RNA-Seq and bioinformatic analysis. Results showed 281 genes were differentially expressed due to γ-EV vs vehicle control, 111 of these genes were common to EV treatment. Most of the genes differentially expressed in response to γ-EV or EV treatment were downregulated vs vehicle control except for genes involved in the processing of mRNA and ribosomal proteins. The top 3 GO categories affected by γ-EV vs vehicle control were monocarboxylic acid metabolic process (gene ratio: 95/1135), fatty acid metabolic process (gene ratio: 71/1135), and brush border (gene ratio: 39/1141). The top 3 GO categories differentially affected by γ-EV vs EV were neuromuscular junction (gene ratio: 11/590), response to metal ion (23/590), and monosaccharide catabolic process (gene ratio: 7/590). KEGG pathway analysis showed that genes involved in glycolysis, gluconeogenesis, carbohydrate digestion and absorption, starch and sucrose metabolism leading to ATP generation were significantly enriched in γ-EV vs EV treated enteroids. In conclusion, the transcriptional changes induced by γ-EV and EV in murine enteroids overlapped significantly. γ-EV diverged from EV by affecting genes involved in carbohydrate metabolism and ATP biosynthesis.

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ASBMB

Abstract 1894

Differential contributions of distinct free radical peroxidation mechanisms to the induction of ferroptosis

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Ferroptosis is a regulated form of cell death that is driven by the peroxidation of polyunsaturated fatty acids (PUFAs). Either restriction of access to cysteine or direct inhibition of glutathione peroxidase 4 (GPX4) induces ferroptosis. Endogenous PUFAs in the human body, such as arachidonic acid, only contain nonconjugated double bonds, and their peroxidation proceeds primarily via the hydrogen-atom transfer (HAT) mechanism. Recently, using a radical clock we developed, we discovered that PUFAs with conjugated double bonds display much higher reactivity toward lipid peroxidation than nonconjugated PUFAs, and their peroxidation proceeds primarily via the peroxyl radical addition (PRA) mechanism. Specifically, conjugated linoleic acid (CLA 18:2) is twice as reactive as nonconjugated linoleic acid (NLA 18:2) while conjugated linolenic acid (CLA 18:3) is over 8 times more reactive than nonconjugated linolenic acid (NLA 18:3). We aim to elucidate the mechanism of CLA oxidation and the roles of the oxidation products in ferroptosis induction. We first demonstrated that CLA 18:3 can induce ferroptosis in cancer cells without other ferroptosis inducers at low mM concentrations and CLA 18:2 more potently potentiates RSL3 (a GPX4 inhibitor)-induced ferroptosis than nonconjugated PUFAs. The PRA mechanism favors the formation of peroxide oligomers and upon C-C bond cleavage, the formation of highly electrophilic a,b-unsaturated aldehydes in comparison with the HAT mechanism. We confirmed the formation of such aldehydes from CLAs at levels much higher than those from NLAs in solution by aldehyde-trapping and mass spectrometry. Lipidomics revealed that conjugated and nonconjugated PUFAs are incorporated into distinct cellular lipid species. We then carried out RNA sequencing to elucidate the signaling pathways mediating CLA-induced ferroptosis and found that protein processing in the endoplasmic reticulum and proteasome are the most significantly upregulated pathways, suggesting increased ER stress and activation of unfolded protein response. Furthermore, using click chemistry, we observed increased protein adduction by oxidized lipids in cells treated with an alkynylated CLA 18:2 probe. These results suggest that PRA much more potently induces ferroptosis than HAT likely due to the preferential formation of electrophilic oxidation products.

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Abstract 1916

Arsenic toxicity on metabolism and autophagy in brown adipose tissue

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Arsenite, a trivalent form of arsenic (As), is an element that occurs naturally in the environment. Humans are exposed to As through consuming As-contaminated drinking water and food, thus As can accumulate in tissues. Arsenite induces oxidative stress that is linked to metabolic disorders such as obesity. Brown adipocytes dissipating heat have emerging roles for obesity treatment and prevention. Therefore, understanding the physiological role of brown adipocytes can provide effective strategies delineating the link between As exposure and metabolic disorders. Our study revealed that arsenite significantly reduced differentiation of mouse brown adipocytes and mitochondrial biogenesis and respiration, leading to attenuated thermogenesis via UCP1 inhibition. Oral administration of arsenite in mice resulted in heavy accumulation in brown adipose tissue (BAT) and suppression of lipogenesis, mitochondrial biogenesis and thermogenesis. Arsenite exposure significantly inhibited autophagy necessary for homeostasis of BAT through suppression of Sestrin2 and ULK1. These results clearly confirm the emerging mechanisms underlying the implications of As exposure in metabolic disorders.

This research was supported by Undergraduate Creative Activities and Research Experience (UCARE) program-scholarship, University of Nebraska ARD/ORED/BIOC grants, Layman awards, Nebraska Tobacco Settlement-Biomedical research enhancement funds and Nebraska Center for the Prevention of Obesity Diseases (NPOD) seed grant from NIH (P20GM104320) to S-H. Ro.

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Abstract 1918

Post-translational regulation of mitochondrial metabolism

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Phosphorylation has long been appreciated to influence mitochondrial metabolism via the regulation of pyruvate dehydrogenase. However, the extent to which phosphorylation broadly influences mitochondrial function remains unclear, despite the presence of multiple protein phosphatases within this organelle. We recently demonstrated that deletion of the mitochondrial matrix phosphatase Pptc7 causes perinatal lethality in mice, suggesting that the regulation of mitochondrial phosphorylation is essential in mammalian development. Pptc7-/- mice exhibit severe metabolic deficiencies, including hypoglycemia, hypoketosis, and lactic acidosis, and die within one day of birth. Biochemical and proteomic approaches revealed that Pptc7-/- tissues have decreased mitochondrial function concomitant with a post-transcriptional downregulation of mitochondrial proteins. Follow up studies have linked this decrease in mitochondrial protein content to excessive mitophagy via the stabilization of two mitophagy receptors, Bnip3 and Nix. Our studies demonstrate that knockout of Bnip3 and Nix rescues the decrease in mitochondrial proteins seen in Pptc7-/- cells, but fails to fully rescue the metabolic defects seen in these cells. These data suggest a multifactorial role for Pptc7 in enabling mitochondrial metabolism, and that the aberrant accumulation of phosphorylation within mitochondria compromises organellar metabolism.

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Abstract 1925

Investigating the conditionally essential role of protein UFMylation in proliferating human cells

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CRISPR-based forward genetic screens can be used to identify the genetic dependencies of proliferating human cells and how these may vary with genotype and lineage. However, although environmental factors also influence gene essentiality, most CRISPR-based screens are performed on cultured cells growing in conventional media that poorly reflect nutrient conditions within the human body. Previously we developed the first systematically designed physiologic cell culture medium, Human Plasma-Like Medium (HPLM), which contains over 70 defined components at concentrations that more closely reflect those in human blood. To test the hypothesis that medium composition can impact gene essentiality, we performed genome-wide CRISPR screens on the K562 chronic myelogenous leukemia cell line growing in either HPLM or RPMI – the conventional medium used to culture blood cells. Analysis of our recently published screen results revealed hundreds of conditionally essential genes that collectively span diverse biological processes. Among the RPMI-essential hits we identified were all known components of UFMylation, a system that attaches UFM1 to proteins. Remarkably, while this post-translational modification (PTM) was initially discovered nearly twenty years ago, the physiologic function of UFMylation remains poorly characterized. To begin to investigate the conditionally essential role of this PTM, we have engineered UFM1-knockout K562 cells and then used short-term growth assays in HPLM versus RPMI to confirm the RPMI-essential phenotype suggested by our screen results. Interestingly, when we asked how loss of UFM1 might affect the cellular metabolome, we observed a boost in the abundances of intermediates along the de novo serine synthesis pathway, suggesting an unforeseen link between protein UFMylation and serine metabolism. Guided by our preliminary data, we are now using tools in molecular and systems biology to determine the contribution of UFMylation to human cell fitness and why the important of this PTM depends on nutrient availability.

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Abstract 1944

Utilization of high fat diets to explore obesity mechanisms in zebrafish

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With cases of obesity and type II diabetes on the rise, it is critical to identify gene-diet interactions that promote these diseases. Type II diabetes is characterized by elevated blood glucose levels and insulin resistance, a state in which the body no longer properly responds to insulin. Insulin resistance is commonly associated with consumption of a high fat diet. While it is known that hepatic fat and glucose metabolism is deregulated in insulin resistance, the molecular underpinnings of this process remain incompletely understood. Our studies aim to develop methods to assess how diet promotes insulin resistance. While zebrafish are commonly used in the field of developmental biology, studies have also highlighted their potential as a tool to study diet-induced obesity. We tested two methods to induced obesity in zebrafish via high fat diet feeding. First, we modified existing protocols for brine shrimp overfeeding. Second, we developed a flake-based diet enriched with lard. We report that both of these diets result in significant weight gain over a period of three to four weeks. Our results suggest that a lard enriched diet elevates blood glucose levels more substantially than brine shrimp overfeeding, highlighting the utility of this diet for use in metabolic studies. Studies investigating how these diets affect hepatic lipid content and expression levels of obesity associated genes are underway. Overall, our studies highlight the utility of zebrafish as a model organism for investigating the complex relationship between genetics, diet, and disease.

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Abstract 1945

17β-estradiol supplementation mitigates effects of diet-induced obesity in zebrafish

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With increasing prevalence of obesity worldwide, it is necessary to understand the molecular mechanisms of this disease. Obesity, a condition of having excess body fat, is associated with insulin resistance and fatty liver disease. Pre-menopausal women are less prone to developing these conditions. It is hypothesized that higher levels of circulating estrogen in pre-menopausal women protects against deregulation of glucose and fat metabolism, though this protective mechanism remains incompletely understood. Zebrafish (*Danio rerio*) are an ideal organism to study obesity mechanisms, as fatty liver and insulin resistance can be induced via high fat diet feeding. Our studies aim to assess the effect of estrogen supplementation on the development of obesity and insulin resistance in zebrafish. We administered a low (50 mg 17β-estradiol/kg feed) and high (250 mg 17β-estradiol/kg feed) dose estrogen treatment to zebrafish consuming a shrimp-based high fat diet. We report that administration of 250 mg 17β-estradiol/kg feed protects against weight gain in diet-induced obesity. Blood glucose data collected at the end of the six-week study indicates that estrogen also protects against rising glucose levels in obesity. These findings are consistent with results observed in human and mouse studies, and highlight the utility of zebrafish as a model organism to investigate cellular mechanisms of metabolic disease. Studies are underway to assess how hepatic expression levels of obesity associated genes are altered by estrogen supplementation.

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Abstract 1958

Sord deletion reduces erythritol synthesis in liver and kidney of sucrose-fed mice

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Serum erythritol is a biomarker of cardiometabolic disease risk and complications. Elevated erythritol can predict T2DM and cardiovascular disease up to 20 years before onset. Excitingly, we have found that sucrose consumption increases plasma and urinary erythritol levels in mice. We hypothesize that erythritol synthesis is a way to dispose of glucose following the consumption of dietary sugars. Little is known, however, regarding the regulation of erythritol synthesis in mammals. Studies *in vitro* have indicated that this reaction may be catalyzed by several enzymes. Of these enzymes, sorbitol dehydrogenase (SORD) is a primary candidate for erythritol synthesis *in vivo*. SORD knockdown reduces intracellular erythritol by 50% in A549 cells. In addition, relative SORD protein expression is high in tissues that contain the highest erythritol content: liver and kidney. The purpose of this study was to determine if Sord deletion blunts sucrose-induced erythritol synthesis in mice. 8-week-old Sord+/+ (WT) and Sord-/- (KO) mice were fed a high-carbohydrate diet with 10% fat-derived calories supplemented with 30% w/v sucrose for two weeks. After two weeks, we measured non-fasted plasma, urinary, and tissue erythritol using GC-MS. Body weight, caloric intake, and blood glucose were also recorded. All data was analyzed by unpaired t-test except tissue erythritol, which was analyzed by two-way ANOVA. As previously demonstrated, 30% sucrose water elevated plasma and urinary erythritol 10-fold above the levels observed in mice fed plain water. Contrary to our expectation, we found no effect of Sord loss on plasma or urinary erythritol levels. There was a significant effect of genotype on tissue erythritol content ($p < 0.01$, two-way ANOVA); after sucrose feeding, KO animals had a 30% reduction in liver and 25% reduction in kidney erythritol compared to WT littermates. We did not observe differences in food intake, body weight, or blood glucose based on genotype. Our findings suggest that SORD contributes to erythritol synthesis *in vivo*, but that erythritol levels can be maintained by alternative enzymes. Interestingly, plasma and urinary erythritol levels were consistent between genotypes despite differences in tissue erythritol. This suggests that circulating erythritol levels may be protected from depletion when sugar intake is high.

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Abstract 1968

Vaping and Cardiopulmonary Fitness: Consequences of Aerobic Exercise

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Introduction: Physical exercise is essential for fitness and health, particularly with modern societal trends toward sedentary lifestyles and obesity. Impact of vaping upon exercise performance remains a topic of significant interest in social media and end users of vape products but has received little attention from scientific research.

Hypothesis: Routine aerobic exercise offers protection from select cardiopulmonary effects of vaping compared to sedentary individuals and may serve as an interventional strategy to ameliorate pathogenesis and/or promote rehabilitation.

Methods: Voluntary running exercise was offered to individually housed young adult C57BL/6 mice using wheels equipped with revolution counters to document performance on a daily basis. Four distinct groups consisted of both males and females as follows: 1) no exercise, no vape; 2) no exercise, vape; 3) exercise, no vape; and 4) exercise and vape. Particular attention was given to gender-specific difference assessments in exercise performance as well as myocardial structure/function.

Results: Mice typically ran from 3 to 12 miles per night resulting in physiologic myocardial remodeling over a 9 week time course. Both genders exhibited progressive diminished exercise performance beginning at approximately 4 weeks of vape aerosol exposure to conclusion of the study. Gender-specific differences between non-vaped versus vape exposed groups were observed in exercise performance, bronchial wall remodeling, and cardiac functional performance. Cardiac functional performance was 1) increased by running in non-vaped mice, 2) diminished by vaping in non-exercised mice, and 3) preserved in females relative to males in vaped mice. Mucin accumulation in airways prompted by vaping was significantly decreased by running in both males and females.

Conclusions: Gender-specific differences in responses were evident in bronchial wall remodeling as well as cardiopulmonary structure and function in vaped mice allowed to exercise. Overall, exercise appears to be beneficial in our vaping-associated pulmonary injury mouse model to reduce pathogenesis and promote cardiac function. Exercise may improve the health of vapers by improving preservation of cardiopulmonary structure/function.

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Abstract 1972

Aryl Hydrocarbon Receptor regulates glucose and lipid metabolism by premature citrus extract on Palmitic acid (PA) -treated HepG2 Cells

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Background: An epidemic of metabolic disorders such as obesity and diabetes is rising dramatically. Aryl hydrocarbon receptor (AHR) plays a crucial role in glucose, lipid, and cholesterol metabolism in the liver. In contrast, the detailed mechanisms for the effect of premature citrus extract (PCE) on AHR in Palmitic acid (PA)-treated HepG2 cells remain unclear. In this study, we aimed to investigate the activation of AHR regulates glucose and lipid metabolism by PCE on PA-treated HepG2 Cells.

Methods: The intracellular lipid accumulation and triglyceride (TG) contents were quantified by Oil-red O staining and TG assay. The glucose uptake was assessed using 2-[N-(7-Nitrobenz-2-oxa-1, 3-diazol-4-yl) amino]-2-deoxy-d-glucose (2-NBDG) assay. Western blot analysis explored the levels of AHR, AKT, GSK3β, AMPK, ACC, SREBP-2, and HMGCR. The potential interaction between the major citrus flavonoids in PCE and the AHR was investigated by molecular docking analysis.

Results: PCE significantly reduced both intracellular lipid accumulation and TG content in PA-treated HepG2 cells. In addition, the PCE showed increased 2-NBDG uptake in an insulin-independent manner in PA-treated HepG2 cells. Notably, PCE improves hepatic glucose and lipid metabolism by activating AHR, confirmed in HepG2 cells. Moreover, the treatment of PCE increases the regulation of key enzymes in glucose and lipid metabolism; and reduces cholesterol metabolism. Furthermore, docking analysis showed that PCE major citrus flavonoids (Hesperidin and Narirutin) bind to the AHR with high binding affinities and could serve as potential AHR activators.

Conclusion: The current findings demonstrated that PCE regulated hepatic glucose and lipid metabolism via activating AHR on PA-treated HepG2 cells. Overall results suggested that PCE could be exploited as a potential candidate for treating diabetes and obesity.

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Abstract 1973

Defining metabolic perturbations in neurological glycogen storage diseases elucidates therapeutic options

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Glycosylation defects are a hallmark of many nervous system diseases. However, the molecular and metabolic basis for this pathology is not fully understood. We recently demonstrated that N-linked protein glycosylation in the brain is critically and directly connected to glycogenolysis. The primary function of glycogenolysis is to liberate glucose-1-phosphate from glycogen. We demonstrated that in addition to glucose, glucosamine is an abundant covalent constituent of brain glycogen. We defined the biosynthetic incorporation of glucosamine into glycogen by glycogen synthase and glucosamine release by glycogen phosphorylase *in vitro* by biochemical and structural methodologies, in situ in primary astrocytes, and *in vivo* by isotopic tracing and mass spectrometry imaging. We established that glycogen-derived glucosamine is a significant source of amino sugars required for protein glycosylation in the brain. Further, we demonstrated that disruption of cerebral glycogen metabolism causes global decreases in free UDP-N-acetylglucosamine and N-linked protein glycosylation using two glycogen storage disease models. These findings reveal a previously unknown yet key fundamental biological role for brain glycogen in protein glycosylation that has direct relevance to multiple human diseases of the central nervous system. Unpublished data will be presented regarding brain monosaccharide metabolism in multiple brain-centric diseases from both mouse models and patient samples.

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Abstract 1989

Stearoyl-CoA desaturase Deficiency Improves Insulin Signaling and Represses De Novo Lipogenesis by Altering Levels and The Fatty Acid Composition of Hepatic Phosphatidylglycerols During High Carbohydrate Feeding

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The increased incidence of obesity, mainly due to de novo lipogenesis, with its complications, such as insulin resistance, type 2 diabetes, cardiovascular disease, liver steatosis or cancer, poses one of the predominant global health threats. Stearoyl-CoA desaturase (SCD), a critical regulator of lipogenesis, catalyzes the synthesis of monounsaturated fatty acids (MUFA), mainly oleoyl- (18:1n9) and palmitoleoyl-CoA (16:1n7), and is elevated in human and rodent obese and insulin resistant states. To broaden our knowledge about the role of SCD in metabolic diseases, we performed *in vivo* investigations using SCD1 liver-specific knockout (LKO) mice that were fed a high carbohydrate low fat diet (HCD) followed by blood and liver lipidomic analysis. All animal studies were conducted in accordance with the Institutional Animal Care and Use Committee guidelines at The University of Wisconsin-Madison. We found out that while the nuclear fraction of the SREBP-1c transcription factor and its lipogenic target genes is decreased in the LKO mice fed a high carbohydrate low fat diet, there was a dramatic increase in the membrane levels and saturated fatty acid composition of phosphatidylglycerol (C16/C18:0-PG). Supplementation of the high carbohydrate diet with oleate in the form of triolein and fed to the LKO mice is sufficient to normalize the levels and fatty acid composition of PG, nuclear SREBP-1c levels and the expression of the lipogenic gene program in both male and female mice. Moreover, the LKO mice had lower adiposity as shown by lower epididymal WAT weight and lower total body weights. Our studies also revealed that the LKO mice present with lower blood glucose levels during an oral glucose tolerance test (oGTT) compared with the control mice despite having similar insulin levels, suggesting that the LKO mice were more insulin sensitive. Our studies suggest that SCD, a regulator of MUFA synthesis, moderates hepatic insulin signaling and SREBP-1c mediated de novo lipogenesis partly by modifying the membrane levels and fatty acid composition of liver phosphatidylglycerols, which likely ameliorated obesity that consequently improved insulin sensitivity in the LKO mice.

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Abstract 2014

Ablation of LFABP (FABP1) leads to altered bile acid homeostasis and hyperplastic white adipose expansion

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Liver fatty acid-binding protein (LFABP) functions in intracellular lipid trafficking in liver and small intestine and is also thought to serve as the major hepatic bile acid (BA)-binding protein. We have recently shown that high fat (HF)-fed LFABP null (LFABP$^{-/-}$) mice display higher abundance and diversity of gut microbiota compared to wild-type (WT) mice[1], likely contributing to the biotransformation of primary to secondary BAs. They have lower circulating BA levels but a higher proportional contribution of secondary BAs. The LFABP$^{-/-}$ mice manifest hyperplastic expansion of inguinal white adipose tissue (iWAT), possibly contributing to their previously described 'metabolically healthy obese' (MHO) phenotype[2, 3]. It has been previously reported that white adipocytes accumulate BA species reflective of those in plasma, have active BA signaling, and that BAs influence adipogenesis[4]. To gain insight into the potential effects of *Lfabp* ablation on BA homeostasis and how it may determine iWAT quality, we performed RNA sequencing analysis in liver and iWAT samples of HF-fed LFABP$^{-/-}$ and WT mice. Pathway analysis of gut microbial species was also performed to better understand how gut microbiota may affect iWAT quality. LFABP$^{-/-}$ mice display decreased expression of hepatic enzymes involved in BA biosynthesis (*Cyp7a1, Cyp39a1, Hsd17b6*) and BA transporters (*Slc23a2, Abca2, Abca6, Abca8b*) in liver, and hepatic BA metabolism pathways trended lower upon *Lfabp* deletion and DIO (FC = −1.04, FDRq = 0.643). Interestingly, these changes in liver are accompanied by a significant and similar downregulation of BA metabolism pathways in iWAT (FC = −1.40, FDRq = 0.124), as also seen by lower transcripts of BA transporters (*Abca6, Abca9*). The LFABP$^{-/-}$ mice have an altered iWAT transcriptomic profile favoring adipogenesis (increased *Esrra, Dusp1, Cdkn1a, Gadd45g, Hr*, and decreased *Fam13a, Cyp2e1*), including enrichment in genes related to selective autophagy and the mitotic spindle *apparatus, both necessary for* efficient white adipogenesis. Further, *Lfabp* deficiency upon DIO upregulates iWAT cholesterol biosynthesis, mTORC1 and MAPK signaling pathways, all of which denote tissue growth. The LFABP$^{-/-}$ gut microbiome, moreover, shows upregulation of lipopolysaccharide (LPS) biosynthetic pathways, while LFABP$^{-/-}$ iWAT displays an increase in the Dectin1-mediated noncanonical NF-κB signaling pathway, possibly secondary to gut microbiota-derived LPS stimulation. In summary, ablation of *Lfabp* results in downregulated BA metabolism in the liver, where it is normally expressed, and in iWAT where it is not. In the gut, higher obesity-associated microbial populations may enhance the conversion of primary

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to secondary BAs, leading to relatively higher secondary BAs in plasma. BAs and LPS are translocated to iWAT, whereby LPS may induce non-canonical NF-κB signaling and downstream PI3 K/AKT/mTOR signaling, potentially resulting in tissue growth through hyperplasia; decreased BA levels have also been shown to induce adipogenesis, perhaps contributing to the hyperplastic expansion of iWAT observed upon deletion of LFABP in intestine and liver. Overall, ablation of LFABP in liver and intestine leads to altered bile acid homeostasis and hyperplastic white adipose tissue expansion.

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Abstract 2063

Reactive-oxygen-species and Cysteine-oxidation Dependence of Tardigrade Cryptobiosis

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The tardigrade, an organism unique in its ability to survive a variety of extreme environmental conditions, is a semi-microscopic invertebrate whose biochemical means of protection may prove fruitful in the development of cancer therapeutics, dry vaccines, and drought resistant crops. The tardigrade protects itself via the formation of a stasis-state in a process known as cryptobiosis which is characterized by an expulsion of internal water content along with other observable physiological changes. Although scientists have identified tardigrade-specific intrinsically disordered proteins that play a role in cryptobiotic processes, more research is necessary for the elucidation of these pathways. Using the model tardigrade *Hypsibius exemplaris*, we provide novel empirical evidence of chemobiosis, the cryptobiosis which results from the direct application of reactive oxygen species (ROS). In conjunction with results from ROS analysis via electron paramagnetic resonance spectroscopy, we postulate that ROS plays an essential role in the cascade of cryptobiosis formation. Using mass spectrometry, diagonal gel electrophoresis, and confocal fluorescence microscopy, we further demonstrate that cryptobiosis is dependent on reversible cysteine oxidation. Overall, this study provides evidence for the role of ROS in mediating cryptobiotic signaling processes via reversible cysteine oxidation.

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Abstract 2091

Living in the cold without brown adipose tissue lipolysis

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Brown adipose tissue (BAT) consumes macronutrients to fuel catabolic processes that generate heat in a process termed non-shivering thermogenesis (NST) making it an attractive target against obesity. NST is mediated by uncoupling protein 1 (UCP1) which is activated and fueled by fatty acids (FAs). These FAs primarily derive from triacylglycerols (TAGs) stored in cytoplasmic lipid droplets that are hydrolyzed by adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL) in a process termed lipolysis. Lipolysis has been considered essential for NST. This dogma was challenged when BAT-specific ATGL knockout mice showed normal NST. HSL exhibits a minor TG hydrolase activity raising the question whether HSL-mediated FA release is sufficient to ignite UCP1 in the absence of ATGL. To test this hypothesis, we generated a mouse model lacking both ATGL and HSL specifically in BAT (BAT-iDAKO). All mouse studies were approved by the Austrian Federal Ministry of Education, Science and Research. BAT-iDAKO mice showed reduced ATGL and HSL protein expression in BAT resulting in ≥60% lower TAG hydrolase activities and a ∼7-fold higher BAT mass. Stressed with cold, BAT-iDAKO maintained euthermia in cold. However, BAT of BAT-iDAKO mice had reduced UCP1 and mitochondrial protein content. Functionally, BAT-iDAKO mice had diminished BAT respiratory capacity and reduced fuel uptake. Instead, we observed increased UCP1 protein content, respiratory capacity, and substrate uptake in inguinal white adipose tissue in BAT-iDAKO. Together, our data show that the lack of both ATGL and HSL results in a dysfunctional BAT. Furthermore, our data suggest that body temperature upon BAT-specific loss of ATGL and HSL is defended by increased white adipose tissue metabolism.

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Abstract 2093

Protein O-GlcNAcylation is Important for Vascular Calcification in Diabetes

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Our previous studies have shown that increased vascular protein O-linked GlcNAc modification (O-GlcNAcylation) promotes vascular calcification in diabetes. In this study, with a loss-of-function approach, we determined whether O-GlcNAcylation is essential for diabetic vascular calcification, and uncovered a novel molecular mechanism underlying O-GlcNAcylation-mediated calcification of vascular smooth muscle cells (VSMC). A new mouse model with inducible SMC-specific deletion of the O-GlcNAc transferase (OGT) was generated by breeding the OGT floxed mice with the inducible SMMHC Cre transgenic mice. Smooth muscle cell (SMC)-specific OGT deletion significantly inhibited protein O-GlcNAcylation and vascular calcification in diabetic mice induced by low-dose streptozotocin. Increase vascular expression of Runx2, the essential osteogenic regulator for vascular calcification, was observed in the diabetic mice, which was markedly inhibited by SMC-specific OGT deletion. With VSMC from OGT deficiency mice, we further demonstrated that OGT deletion downregulated Runx2 expression and Runx2-induced VSMC calcification. A direct O-GlcNAc modification on Runx2 was uncovered by immunoprecipitation analysis, which was abolished in the OGT-deficient VSMC. OGT modification on Runx2 Threonine 412 (T412) was identified with a serial of Runx2 truncation mutants and point mutations on putative O-GlcNAcylation sites. Importantly, inhibition of Runx2 O-GlcNAcylation on Runx2 T412 abolished Runx2-induced VSMC calcification, which was associated with inhibition on Runx2 transcription activity and Runx2 binding with its key co-factors. In summary, our studies have demonstrated a novel causative link between protein O-GlcNAcylation and VSMC calcification, which is important for vascular calcification in diabetes. With an array of comprehensive biochemical, molecular and cellular approaches, we have uncovered an essential role of O-GlcNAc modification on Runx2 in promoting Runx2 osteogenic transcriptional activity and Runx2-induced VSMC calcification.

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ASBMB

Abstract 2118

Cannabidiol affects differentiation of 3T3-L1 cells into mature adipocytes

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Many phytocannabinoids, compounds derived from the *Cannabis sativa* plant, have been shown to have therapeutic properties. One intensely studied phytocannabinoid, cannabidiol (CBD), has a wide range of pharmacological effects and has postulated anti-adipogenic effects through its interaction with the CB1 endocannabinoid receptor. To test the potential anti-adipogenic effects of CBD, we looked at how a low (0.2 uM; low [CBD]) and a high (20 uM; high [CBD]) concentration of CBD affected the proliferation, differentiation, and expression of genes involved in triglyceride synthesis in 3T3-L1 cells—a murine fibroblast cell line that can differentiate into a mature adipocyte phenotype. CBD was added to the cells either during the expansion (growth) phase or during the differentiation phase (post-confluence). Differentiation was stimulated through addition of insulin, IBMX, and dexamethasone. After differentiation into mature adipocytes, lipid droplets were stained with Oil Red O (ORO), measurements of cell size (area) were performed using ImageJ software, and ORO was extracted and quantified to determine total lipid deposition. Additionally, RNA was extracted for quantification of gene expression via RT-PCR. The effect of CBD on proliferation and viability of cells was tested during the expansion process using an MTS assay and the Trypan blue method. Treatment with the high dose of CBD (20 uM) significantly decreased 3T3-L1 proliferation and cell viability at 24 h, while the low dose (0.2 uM) exhibited no difference compared to control. Interestingly, when cells were treated with the high [CBD] during the differentiation phase there was a complete inhibition of cell differentiation, while treatment during the expansion phase resulted in a significantly decreased number of differentiated cells compared to control and the low CBD dose, but the few differentiated cells were not different in size (area) compared to control cells. As expected, total lipid deposition was decreased when cells were treated with the high [CBD] as this is directly related to the decrease in cell differentiation. When added during the expansion phase, low [CBD] resulted in adipocytes that were not significantly different from control cells in % differentiation and total lipid deposition but that were significantly smaller. Of the genes involved in triglyceride synthesis, diglyceride acyltransferase (DGAT) and glycerol-3-phosphate acyltransferase (GPAT) both exhibited significant decreases in expression when cells were treated with CBD. Addition of both the high and low [CBD] during the differentiation period decreased DGAT expression compared to control cells (2^ddCt), while GPAT expression was only decreased in cells treated with the high [CBD]. Our results suggest that CBD affects proliferation and differentiation of the 3T3-L1 adipocyte model with some of these effects being related to a decrease in expression of the genes necessary for triglyceride synthesis and lipid deposition. The potential anti-adipogenic effects of CBD warrant further investigation, with particular emphasis on concentrations that are physiologically achievable in humans.

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Abstract 2130

Characterizing cancer cell metabolic adaptation to mitochondrial complex II deficiency

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Perhaps the only feature universal among cancers is uncontrolled cellular proliferation, a process which imposes specific metabolic demands on proliferative cells. These demands—although incompletely understood—are often targeted for cancer treatment, but a major hurdle for successful therapies is that cancer cells often exhibit metabolic adaptations from their host tissue which allows them to grow quicker, tolerate harsh tumor microenvironments, metastasize, and evade host defenses and therapies. One major roadblock in our mechanistic understanding of this process is a lack of defined model systems for clinically relevant cancer cell metabolic adaptation. In a recent effort to understand how cells with oncogenic defects in mitochondrial complex II (also called succinate dehydrogenase, SDH) meet their anabolic demands, our lab discovered that cells deficient in mitochondrial complex II gradually improve their proliferation rate by progressively downregulating mitochondrial complex I expression and activity over successive passaging, which we found serves to improve their metabolic function. However, the mechanisms by which these cells adaptively decrease mitochondrial complex I expression to accomplish this metabolic change is unknown. Here, we characterize adaptation in cancer cells following SDH disruption using a time course of cell proliferation assays, metabolomics, transcriptomic and proteomic measurements of complex I subunits, and mitochondrial DNA quantification assays. Overall, our results reveal complex and progressive changes to complex I subunits across levels of biological control in SDH-null cells, suggesting mechanistic possibilities for complex II-deficient cancer adaptation and serving as a basis for identifying the molecular determinants of metabolic adaptation during cancer progression.

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Abstract 2155

When an oncometabolite isn't an oncometabolite: studies of L-2-hydroxyglutarate metabolism in Drosophila

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The enantiomers of 2-hydroxyglutarate (2HG) have emerged as potent regulators of metabolism, chromatin modifications, and cell differentiation. While these compounds are associated with tumor metabolism and commonly referred to as oncometabolites, neither D-2HG nor L-2HG are tumor specific and both compounds are synthesized in a diversity of healthy cell types. Furthermore, hypoxia was recently found to induce L-2HG synthesis in mammalian cells, indicating that this compound serves a normal metabolic purpose in animals. Consistent with this hypothesis, we discovered the fruit fly *Drosophila melanogaster* synthesizes millimolar concentrations of L-2HG during normal juvenile growth. Drosophila L-2HG production is largely derived from glucose, dependent on a metabolic program that mimics the hallmark characteristics of the Warburg effect, and results from activation of the Drosophila Estrogen-Related Receptor, which promotes L-2HG synthesis by up-regulating Lactate Dehydrogenase (Ldh) gene expression. As a result, high levels of larval LDH activity directly synthesize L-2HG from 2-oxoglutarate for the duration of juvenile growth. We have also determined that L-2HG levels are controlled by activity of the mitochondrial citrate transporter (MCT). In humans, mutations in the mitochondrial citrate transporter (MCT) induce a rare genetic disorder known as combined D-/L-2HG aciduria, which is characterized by increased accumulation of both 2HG enantiomers. The mechanism by which MCT deficiency leads to D- and L-2HG accumulation, however, remains unknown. By examining MCT mutations in Drosophila, we have determined that this transporter controls L-2HG accumulation by regulating glycolytic flux. Overall, our studies establish the fly as a powerful genetic system to dissect the conserved molecular mechanisms that control L-2HG accumulation in animal cells.

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Abstract 2168

Analysis of the antioxidant power of plant secondary metabolites from *Acacia rigidula* benth extracts

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Ruby Ynalvez

Oxidative stress is known to play an important role in the development and worsening of neurodegenerative and cardiovascular disease. In particular, the imbalance of the steady-state oxidation-reduction processes caused by the overproduction of reactive oxygen species (ROS) can lead to lipid peroxidation in lipid rich organs such as the heart, brain, kidney, and liver. Recent studies on the medicinal properties of plant secondary metabolites have reported the genus Acacia to have antioxidant activities (AOA), highlighting the need to explore new sources of antioxidant compounds that can be used as models for future antioxidant drugs. Here we report the role of solvent in the extraction of phenolic compounds and the antioxidant activities of three different solvent extracts of *Acacia rigidula* benth leaves using four antioxidant assays: ABTS, DPPH, FRAP, and the ferric thiocyanate lipid peroxidation assay. We prepared three solvents extracts using 50% Ethanol, 80% Methanol, and a water, acetone, methanol solution (2:2:6) to quantitatively determine the highest total phenolic content (TPC) yield. All assays were performed in three replicates, in quadruplets. The 50% ethanol and 80% methanol extracts with concentrations of 150 and 250 ppm displayed the highest AOA from all three extracts in the ABTS and DPPH assays. These concentrations showed no significant difference from that of the positive controls gallic and ascorbic acid ($p > 0.05$). For the Ferric Thiocyanate assay, there was no significant difference in AOA among the three extracts ($p > 0.05$). Interestingly, our *A. rigidula* extracts had a higher peroxidation inhibition percentage (PI%) than the positive controls. All three extracts were significantly higher than the gallic acid positive control ($p > 0.05$). The FRAP assay showed that there was a significant difference ($p > 0.05$) between the Gallic Acid Equivalents (GAE) produced by the six different concentrations used (5–70 μg/mL) as reported by a Tukey test. In conclusion, we show that *A. rigidula* benth has antioxidant activities comparable, and sometimes higher than commonly used antioxidants like gallic acid when using extract concentrations between 50 and 250 μg/mL. Our study also reveals that there is no significant contribution coming from the solvent when comparing AOA, suggesting that the AOA is coming not only from the phenolic compounds extracted but by a combination of secondary metabolites present in the *A. rigidula* extracts.

College of Arts and Sciences and the Office of Graduate School at Texas A&M International University.

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Abstract 2170

Cerium(III) as a Supplement to Enhance *Pseudomonas putida* KT2440 Growth and Microbial Transformation of Aromatic Hydrocarbons

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Dominique Williams, Donte' Thompson

Biofilms are communities of surface-attached bacterial cells encased in an exopolymeric matrix. In this state, they are more resistant to antimicrobial treatment and can have adverse effects in medical, agricultural, and industrial settings. Whereas, as biocatalysts, immobilized biofilms from nonpathogenic bacteria enhance their performance and stability in catalysis. Unfortunately, there are several challenges when using bacteria in organic transformations due to their complex cellular chemistry. The main goal of our research is to use cerium(III) to control the adhesion, viability, and activity of *Pseudomonas putida* KT2440 to improve biocatalysis. Trivalent lanthanide metals were discovered to serve regulatory roles in some bacterial catalytic processes, including those of *P. putida*, a non-infectious Gram-negative bacterium. In our studies, we investigated how Ce(III) influences biofilm formation and cell growth in the presence of toxic levels of aromatic compounds. Two of the aromatic compounds tested were the metabolic intermediates catechol and vanillin. Crystal violet binding assay was used to assess biofilm formation, and a cell growth assay and colony forming unit assay were used to assess planktonic cell growth and cell viability. Our studies demonstrated that Ce(III) improved biofilm formation, planktonic cell growth, and cell viability in the presence of aromatic compounds with glucose as the carbon source. When glucose was replaced with citrate, preferential biofilm formation and planktonic cell growth was not observed in *P. putida* with Ce(III). Thus, Ce(III) promoted biofilm growth, planktonic cell growth, and cell viability, and was dependent on the carbon source used to aid growth. We further investigated how Ce(III) influences the depletion of benzoate in catalysis.

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Abstract 2187

Phenylketonuria (PKU): A Review of the Diagnosis and Treatment of PKU

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Phenylketonuria (PKU) is an autosomal recessive disorder affecting the metabolism of phenylalanine. Mutation in the phenylalanine hydroxylase (PAH) gene disrupts the enzyme used to break down phenylalanine amino acid into tyrosine and melanin. As a result, phenylalanine gets accumulated in the body which leads to extreme elevations of phenylalanine concentrations in the blood, characterizing a condition known as hyperphenylalaninemia. Phenylalanine gets deaminated to form phenylpyruvate and other phenylketones which are excreted in the urine resulting in phenylketonuria. Currently, there is no known cure for PKU. Early detection is crucial because if left untreated, the condition can negatively affect the brain. Prolonged exposure to high levels of phenylalanine can lead to severe intellectual disability, behavioral or psychiatric difficulties, motor issues, and lightening of the skin, eyes, and hair pigmentation. The objectives of this research are to (1) analyze how PKU is diagnosed and (2) determine the effects of current treatments. Electronic databases such as JSTOR and EBSCOhost were used to search for peer-reviewed articles using keywords such as "Phenylketonuria," "phenylalanine metabolism," and "treatments and management," related to PKU. Twenty articles were selected based on their relevance to the topic and were used to address the effects the treatments had with affected patients. Studies reveal modern medicine allows neonatal tests to be done by collecting a drop of blood by pricking the bottom of the newborn's heel to be performed as early as 24 hours after birth for early detection of PKU. Treatments include (1) dietary management by controlling the amount of natural protein intake, (2) pharmaceutical agents such as pegvaliase and sapropterin to reduce blood Phe concentrations, (3) synthetic biotics such as *E. coli* strain SYNB1618 to degrade Phe from the gastrointestinal tract, and (4) tolerance build up in infants through the titration of breast milk with blood phenylalanine levels. Advancements in technology can be integrated in the diagnosis and treatment for PKU by (1) genetically modifying individuals with CRISPR technology to fix defects in the genetic components, and (2) developing novel pharmaceuticals that reduce adverse effects caused by the current medications available.

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Abstract 2207

Fructose induces TNFa expression in a cell culture model of fatty liver and contributes to lipotoxicity without excess lipid accumulation compared to glucose-fed cells

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Jennifer Lewis, Sarah Oppelt

Roughly one third of the world's adult population is affected by metabolic-associated fatty liver disease (MAFLD). MAFLD is characterized by hepatic lipotoxicity, chronic inflammation, hypertriglyceridemia, and insulin insensitivity. MAFLD prevalence doubled during the first decade of the 2000s. This increase in MAFLD development has followed the trend of increased dietary fructose in the developed world. There is mounting evidence that overconsumption of fructose contributes to MAFLD by increasing de novo lipogenesis and driving inflammation. Biopsy samples from patients and experiments with animal models show infiltration of fatty liver by immune cells and increase in proinflammatory signals, such as TNFα and IL-1β. However, the initiating events that lead to the proinflammatory signaling and inflammation in MAFLD development remains an open question. Cell culture models of fatty liver have shown that fructose exposure promotes de novo lipogenesis, excess lipid accumulation, and oxidative stress. Here, we quantified lipid accumulation, mitochondria function, oxidative stress, and expression of proinflammatory cytokines in HEPG2 cells exposed to physiologically normal glucose levels (5 mM), excess glucose (25 mM), and excess fructose (25 mM). Lipid accumulation was quantified using the Oil Red O stain for neutral lipids. Mitochondria function was assessed by comparing the activity of mitochondria dehydrogenases with the WST-8 assay. qPCR was used to measure expression of glutathione peroxidase and ASC as indicators of oxidative stress, and TNFα and IL-1β as markers of proinflammatory signaling. HEPG2 cells exposed to excess glucose or fructose showed similar levels of lipid accumulation and cell viability. However, exposure to fructose decreased mitochondria function, increased oxidative stress, and increased expression of proinflammatory cytokines before cells became lipotoxic. This suggests that total lipid accumulation is not sufficient to cause lipotoxicity, but instead, metabolically-stressed hepatocytes are promoting inflammatory responses that then lead to lipotoxicity. Understanding the original drivers of inflammation is important for proper intervention and treatment of MAFLD.

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ASBMB

Abstract 2220

Progression from steatosis to steatohepatitis correlates with immune cell infiltration and activity at early stages of liver disease in a aldoB-/- mouse model of metabolic associated fatty liver disease (MAFLD)

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Aaron Valdez, Kathryn Satko, Sahrah Rajeh, Dean Tolan, Sarah Oppelt

Metabolic-associated fatty liver disease (MAFLD) affects 1 in 3 people in the US and is a healthcare burden on the order of billions of dollars. MAFLD is characterized by pathological lipid accumulation in the liver, excess production of triglycerides and cholesterol, and export of LDL particles that raise serum lipid levels, liver fibrosis, chronic inflammation, and insulin resistance. Diagnosing MAFLD prior to fibrosis is crucial for successful intervention and prevention of advanced liver disease and other metabolic complications. However, common MAFLD models take months to develop pathology. Increased hepatic lipogenesis can also be achieved utilizing the resulting accretion of fructose-1P caused by fructose overconsumption. Previous studies demonstrate that mice lacking the enzyme ketohexokinase, responsible for phosphorylating fructose to fructose-1P, do not accumulate fructose-1P and are resistant to developing MAFLD. Alternatively, mice deficient in aldolase B, which cleaves fructose-1P, will readily accumulate excess intracellular fructose-1P and show exaggerated hepatic ballooning, macrovesicular lipid droplets, and immune cell infiltration. Here we show that the fructose-sensitive aldoB-/- mouse develops MAFLD pathology similar to conventional models within a week, compared to the conventional models, which take months. In this study, aldoB-/- mice, and their WT counterparts, were fed fructose-free diets, or regular chow (0.75% fructose) diets, for 1, 3, or 7 days. Histological analysis of cytocellular architecture, quantification of total lipids, and characterization of immune cell activity were used to characterize development of steatosis, inflammation, and collagen deposition. We found that aldoB-/- mouse livers develop major steatosis, tissue disorganization, inflammation, and fibrosis within 24 hours of fructose exposure. Due to its sensitivity to developing MAFLD in response to small quantities of dietary fructose, the aldoB-/- model allows research of early time points of MAFLD development more efficiently. Because of this, the aldoB-/- model can aide in identifying biomarkers for MAFLD while the condition is still reversible in patients.

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Abstract 2221

An *in vitro* model of metabolic dysfunction in bipolar disorder

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Aislinn Williams

Though bipolar disorder (BD) is common, it remains one of the most severe, and least treatable, psychiatric illnesses. Poor understanding of disease etiology has led to a lack of treatment targets, greatly hindering progress toward new therapies. However, a number of studies using a variety of techniques—from MRS imaging to metabolomics to genetic analyses—have identified changes in cellular energy metabolism and hallmarks of mitochondrial dysfunction in patients with BD. We therefore hypothesize that mitochondrial dysfunction may be a key potential therapeutic target in BD. To test our hypothesis, we generated induced pluripotent stem cells from three patients with BD and three healthy controls, which we then differentiated into cortical glutamatergic neurons for phenotypic assessment. Consistent with clinical data, we found that BD neurons cultured *in vitro* displayed hallmarks of mitochondrial dysfunction including severe neuritic beading, reduced mitochondrial membrane potential, and lower intracellular ATP concentration compared to control neurons. Surprisingly, we also found that BD stem cells are more unstable than control stem cells, and neurons differentiated from BD stem cells develop more quickly, reliably producing neurites days before their control counterparts. Our results suggest that stem cell-derived neurons present with a disease-relevant phenotype, allowing us to further investigate the molecular etiology of BD *in vitro*, as well as providing a means to screen potential treatments.

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Abstract 2247

The effect of copper on yeast lifespan

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Mitochondria are essential organelles in both yeast and human cells due to their role in metabolism, ATP production via the oxidative phosphorylation (OXPHOS) pathway, and other regulatory cellular processes. Using yeast as a model organism to study mitochondrial function in a chronological lifespan assay allows experiments to be conducted over a shorter timeframe and allows for connections to be made to human cells. Our aim is to investigate how exogenous copper in the mitochondria of yeast affects the production of reactive oxygen species (ROS), protein expression, and enzyme activity during yeast lifespan. Small amounts excess copper added to growth media (0.25 mM copper sulfate in restricted nutrient media) extend yeast chronological lifespan, but yeast lifespan is reduced when added copper levels are increased to 2.0 mM copper sulfate or higher. These results indicate that low levels of exogenous copper in the media is beneficial for yeast in these restricted media conditions. To extend these findings, we assessed how added copper changes mitochondria within these cells over the course of the yeast lifespan (14 days growth). Using MitoTracker Green and fluorescence detection we showed an increase in mitochondria in copper treated cells. This is consistent with previous studies showing mitochondria in mammal and yeast cells contain a labile copper pool located in the matrix, which is used in the metalation of the copper containing enzyme of the OXPHOS pathway Complex IV, Cytochrome C Oxidase (CcO), and superoxide dismutase (Sod1p). Our most recent work focuses on assessing CcO protein complex expression during yeast lifespan, specifically looking at cytochrome c oxidase subunits using western blotting; and assessing Sod1p activity using in-gel activity assays. The results of this research allow us to better understand the role of copper in mitochondrial activity across the lifespan of yeast.

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Abstract 2249

Metabolic flexibility powers up neutrophil functions

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Neutrophils are the most abundant leukocytes at the front line of innate immunity. Upon sensing signals associated with infection, neutrophils rapidly turn on a series of functions critical for host defense. However, how neutrophils utilize metabolism of various nutrients to power up these immune functions, which are associated with substantial metabolic demands, remains poorly understood. We quantitatively characterized the metabolic rewiring in human neutrophils upon activation by a variety of stimuli using metabolomics and isotopic tracing approaches. We found that upon activation, neutrophils undergo rapid shifts in both nutrient preference and metabolic flux distribution among different pathways. Regarding metabolic sources, neutrophils shift from relying on the combined contributions of internal glycogen storage, extracellular glucose, and other sources, to primarily utilizing extracellular glucose upon stimulation. Regarding metabolic fate, neutrophils undergo a switch from glycolysis-dominant metabolism to a unique metabolic mode termed pentose cycle, where all glucose-6-phosphate is shunted into the oxidative pentose phosphate pathway (PPP), and the net flux through upper glycolysis is reversed to allow efficient carbon recycling. This switch to pentose cycle maximizes NADPH yield and is quantitatively coupled to the oxidative burst. These rapid changes in nutrient utilization and metabolic flux distribution are mediated by fast-acting molecular mechanisms, including small metabolite-driven regulations, phosphorylation of key enzymes, and regulation of transporters. The substantial metabolic rewiring upon activation demonstrates neutrophils have remarkable metabolic flexibility, which we found is essential for neutrophil functions. Limiting neutrophils' flexibility to switch to pentose cycle greatly suppresses effector functions including oxidative burst, neutrophil extracellular trap release, and pathogen killing. And by perturbing neutrophils' utilization of different nutrients, we found different neutrophil functions have function-specific nutrient dependences. Overall, this work revealed important metabolic underpinnings enabling neutrophils functions upon activation.

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ASBMB

Abstract 2253

Hepatic FXR and SHP depletions induce sex differences in bile acids metabolism

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Christopher Gaulke, SayeeSayeepriyadarshini Anakk

Once reabsorbed by the distal intestine mucosa, bile acids (BA) are transported via the portal vein to the liver where they exert negative feedback on their own synthesis via the activation of the nuclear receptors Farnesoid-X-receptor and Small Heterodimer Partner (FXR and SHP, respectively) in hepatocytes. Our aim was to decipher how specific hepatic FXR/SHP depletions impact BA composition, enterohepatic circulation and its cross talk with the gut microbiota. Total BA were measured in portal and systemic blood, but also intestinal, hepatic tissues and feces from 3–6 months-old fed female and male wildtype (WT) and LDKO (liver specific FXR/SHP depletions) mice. In addition, upper GI transit was investigated in overnight fasted WT and LDKO mice by measuring methylene blue dye progression. Compared to WT, portal BA were found higher in LDKO mice which exhibited sex-differences; females displayed higher portal but also systemic levels in contrast to males, without change however in hepatic and intestinal levels. Moreover, fecal BA levels were higher in females suggesting increased fecal excretion despite of similar intestinal transit time. We will be analyzing gut microbiota composition under these various conditions to study the impact of sex, BA composition on the microbiome. Together these data highlight altered BA absorption at the intestinal and hepatic levels of LDKO mice which suggest differences 1) in bile acids composition resulting from sex-specific gut microbiota, and 2) in hepatic BA uptake depending not only on FXR/SHP but also on sex hormones. Similar investigations in ovariectomized LDKO and wild type mice, associated to the characterization of the gut microbiota and bile acids compositions in the different models, will help us to better understand the role of hepatic FXR and SHP in bile acids circulation between intestine and liver.

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Abstract 2257

Examining the Role of POLRMT Loss in the Mechanism of Action of Novel ClpP-activators

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Sabrina Daglish, Emily Fennell, Laura Herring, Edwin Iwanowitz, Ekhson Holmuhamedov, Lee Graves

Small molecule activators of the mitochondrial protease ClpP, including ONC201 and structural analogs (TR compounds) are an emerging class of anticancer compounds. We recently showed that activation of ClpP with TR compounds (Madera Therapeutics) resulted in growth inhibition in triple negative breast cancer (TNBC) cell lines and *in vivo* models. We observed downregulation of mitochondrial (mito) transcription machinery, including the mitochondrial DNA-directed RNA polymerase (POLRMT). Inhibitors of POLRMT (e.g., IMT1) exert anticancer responses similar to those observed with ClpP activators including inhibition of cell growth, loss of mtDNA, and inhibition of oxidative phosphorylation. The objective of this study was to identify biochemical similarities between ClpP activation and POLRMT inhibition to determine whether POLRMT loss plays a significant role in the mechanism of TR-57 induced growth inhibition. The TNBC cell line, SUM159 (wildtype and ClpP knockout) was utilized in this study. Cell viability was assessed by Hoechst stain and cell counting performed with the Celigo imager. mtDNA loss was measured by confocal microscopy. Changes in specific protein levels were assessed by immunoblotting. q-PCR of mitochondrial genes ND1, ND6, and COII was used to measure changes in mito transcription. The time and dose-dependent effects of TR-57 and IMT1 on multiple phenotypic and biochemical parameters in TNBC cells were compared. TR-57 more rapidly inhibits cell proliferation at lower concentrations than IMT1. Dose-response curves in WT SUM159 s showed that IMT1 has an IC50 of ~100 nM after 96 hours of treatment whereas TR-57 was previously shown to have an IC50 of 14 nM. Since both IMT1 and TR-57 are predicted to block POLRMT activity, we compared the effects of IMT1 and TR-57 on mito transcript levels, expression of mitochondrially encoded proteins, and mtDNA content. Quantitative PCR data showed that mito transcription was paused after 48 and 24 hours with TR-57 and IMT1 treatment respectively. Furthermore, inhibition of mito transcription was supported by loss of proteins involved in oxidative phosphorylation, such as COXI, which was depleted in a time-dependent manner with TR-57 and IMT1 treatment. Interestingly, levels of proteins important for initiation and elongation of mito transcription (TFAM, TUFM) strongly decreased after TR-57 and IMT1 treatment, while others such as MRPL12 and POLRMT decreased only after TR-57 treatment. Since ClpP activators induce the integrated stress response (ISR), we compared the effects of TR-57 and IMT1 on ATF4 expression. Immunoblotting showed that TR-57 induced ATF4 expression, but IMT1 did not, indicating the ISR is triggered by ClpP activation through a POLRMT-independent

mechanism. Finally, loss of mtDNA was observed with TR-57 and IMT1 treatment after 48 and 24 hours respectively, suggesting that both compounds produce similar inhibitory effects on mito transcription and loss of mtDNA. In summary, our data shows that ClpP activation leads to POLRMT loss, resulting in biochemical similarities to the POLRMT inhibitor IMT1. While ClpP activation leads to inhibition of mito transcription, decreases in mitochondrially encoded proteins, and loss of mtDNA (like IMT1), we observed other TR-57-specific effects on cellular function (e.g., ATF4 induction). Further studies will determine whether inhibition of mito transcription is essential for prevention of cell growth observed with ClpP activators.

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Abstract 2264

Metabolic reprogramming by caloric restriction drives gene expression and lipid composition changes in the aging brain

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Mac Anderson, Josef Clark, Rozalyn Anderson

Advanced age is the largest risk factor for chronic diseases, including cancer, diabetes, cardiovascular disease, and neurodegenerative disorders. Brain aging and neurodegeneration are particular concerns, responsible for an ever-growing healthcare burden and lacking effective treatments. Understanding the processes by which aging increases vulnerability to diseases such as dementia is therefore imperative. Caloric restriction (CR), without malnutrition, is a nutritional intervention that delays aging and age-related diseases, including neurodegenerative diseases. While the specific mechanisms responsible for the effects of CR are still incompletely understood, CR induces substantial reprogramming of cellular and organismal metabolism, suggesting that a close examination of metabolism-associated factors in the brain might yield insight into neurodegenerative disease development risk with age. Lipids are a key component of metabolism, serving as substrates from which to derive energy, the primary structural components of the cellular lipid bilayer, and signaling agents. Most of the mass of the brain is comprised of lipids, and the brain relies on specific aspects of lipid metabolism for functions such as synaptic transmission and myelination, in addition to normal cell function. And yet, lipids are an underexplored area of aging brain metabolism. Lipid composition changes due to age in brain are not comprehensively described, nor is the landscape connecting age-related changes in gene expression to lipid metabolism. Emerging evidence suggests that changes in lipid metabolism may be key players in both brain aging and the subsequent development of neurodegenerative diseases, and so a systemic picture of how regulatory networks at the gene level might drive lipid composition changes with age would provide a critical framework for understanding how lipids contribute to aging brain and related diseases. To assess gene expression and lipid composition across aging, we profile brain tissue from mice at three different ages (10, 20, and 30 months of age) using RNAseq and mass spectrometry-based lipidomics. We also examine the effect of CR by profiling tissue from mice of the same ages but maintained on a 25% calorically restricted diet from two months of age. As expected, we find substantial remodeling of the transcriptome due to CR, with engagement of metabolic pathways most prominent in the comparison of 10 month old and 30 month old animals. Lipid profiles indicated an increase in various ceramide species with age, a change that is opposed by CR. Finally, we use clustering and gene co-expression analysis to identify gene sets that correlate with lipid profiles, identifying sets of genes that may regulate lipid composition changes due to the delayed aging intervention of CR.

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Abstract 2302

Defining the metabolic contributions of mitochondrial function during cell proliferation

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Mitochondria are eukaryotic cell organelles that have principal roles in cell metabolism, anchored in part by their unique capabilities afforded by the electron transport chain (ETC) and tricarboxylic acid (TCA) cycle. While the conventional role of the mitochondrial ETC is to generate a membrane potential for ATP generation, additional metabolic contributions of mitochondria beyond ATP synthesis have recently come into focus. Prior studies from us and others have determined that inhibition of mitochondrial ETC complex I (CI) causes dose dependent cell proliferation defects *in vitro* and *in vivo*, which correspond to aspartate depletion and are ameliorated by aspartate restoration. While these findings demonstrate that aspartate production is a critical output of mitochondrial metabolism, it is still unclear if disruptions to other aspects of mitochondrial metabolism similarly mediate their effects on cell proliferation through changing aspartate availability and what aspect(s) of aspartate limitation ultimately cause cell proliferation defects. Here, we describe recent work from our group demonstrating that impairments to succinate dehydrogenase (SDH), an enzyme complex with functions in both the TCA cycle and ETC, has distinct metabolic consequences compared to impairments in other ETC complexes, but ultimately causes aspartate dependent proliferation defects. Interestingly, SDH complex subunits are tumor suppressors, and we find that SDH deficient cancer cells adapt by suppressing CI activity, which alters the mitochondrial redox status to promote alternative aspartate synthesis pathways. By comparing the proliferative and metabolic consequences of inhibition of CI, SDH, or both across environmental conditions, we find we can uncouple many of the downstream metabolic effects of mitochondrial inhibition from cell proliferation, but that the effects on aspartate levels remain a unifying determinant of cell proliferation across mitochondrial impairments. Since aspartate is not only necessary for cell proliferation by supporting protein synthesis, but also for synthesis of asparagine, arginine, and both pyrimidine and purine nucleotides, we next investigated how aspartate limitation mediates its antiproliferative effects. We tested how supplementation with aspartate fates changes the efficacy of mitochondrial inhibitors on cell proliferation and found that, while aspartate fate supplementation can promote partial resistance to mitochondrial inhibitors, it does so by sparing the consumption of aspartate towards those fates and aspartate levels still set the proliferation rate in conditions of mitochondrial inhibition. Collectively, these studies highlight to critical role of mitochondrial metabolism in enabling cell proliferation through supporting aspartate production and

suggest potential therapeutic approaches to alter aspartate levels to modify cell function in relevant disease states.

We acknowledge support from the National Institute of General Medical Science (NIGMS; R35GM147118 and T32GM095421), the National Cancer Institute (NCI; R00CA218679 and U54CA132381), and the Andy Hill Cancer Research Endowment.

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Abstract 2324

Probing electrostatic interactions in the Fo motor of *Escherichia coli* ATP synthase

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ATP Synthase is a molecular motor that utilizes a rotary mechanism to synthesize adenosine triphosphate (ATP), the fundamental energy currency of life. The torque for this mechanism is generated in the membrane-embedded Fo motor where protons flow down the electrochemical gradient through two half channels. In *E. coli*, the Fo motor is composed of a c10 ring (rotor) alongside subunit a (stator), and the H+ exit half channel is located at this rotor-stator interface. The mechanism by which proton translocation is converted into torque on the c-ring is not fully understood. Previous work has suggested that conserved residues aAsp92, aGlu196, and cArg50 in the proton exit pathway are important for proton transport and possibly for torque generation. To investigate the roles of these residues, we generated 28 substitution mutants and assayed their *in vitro* ATP synthesis, H+ pumping, and H+ permeability activities as well as the ability of mutants to carry out oxidative phosphorylation *in vivo*. Mutations of aGlu196 caused only mild effects on proton pumping, while moderately inhibiting ATP synthesis. These results indicate that aGlu196 is likely not interacting with cArg50 but do suggest that it may have a greater role in ATP synthesis than in H+ pumping. In contrast, mutations of aAsp92 were not well tolerated, and mutations that reverse the charge of cArg50 caused a substantial defect. Interestingly, alteration of charge density at the C-terminus of subunit a, which also lies in the exit channel, was able to rescue this defect, suggesting an electrostatic interaction between cArg50 and subunit a. These results begin to uncover a novel rotor-stator interaction in the H+ exit channel that may contribute to H+ translocation and torque generation.

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Abstract 2345

Physiological Characterization of Tardigrade Cryptobioses

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Tardigrades are eight-legged semi-microscopic invertebrates renowned for their ability to survive a wide array of stressful environmental conditions, including the vacuum of space and being shot out of a gun. The ability of tardigrades to survive such extreme conditions is largely attributable to their ability to enter a quasi-stasis state, termed cryptobiosis, wherein they reduce their metabolism to near undetectable levels. While the ability of tardigrades to survive environmental stress is well documented, there is little work investigating differences in the concentration dependence of cryptobiosis induction or the differences in physiological changes brought about by that induction. Herein, the concentration dependence of cryptobiosis induction in the tardigrade *Hypsibius exemplaris* in response to osmotic (sucrose and CaCl2) and chemical (H2O2) stressors is probed. Further, the different physiological changes occuring in response to osmotic, chemical, and cryogenic stress are documented using confocal microscopy. We report that the concentration of applied stressors has a significant effect on the induction of cryptobiotic states and the survival of tardigrades and that tardigrades demonstrate apparent physiological differences across stressors. This works provided a basis for future investigations aiming to probe tardigrade cryptobioses.

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Abstract 2373

In-silico Analysis of Iron Metabolism in Ovarian Serous Cystadenocarcinoma

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Introduction and Objective Iron is an essential element with a wide range of biological roles, with many being linked to tumor metabolism and carcinogenesis. While key iron-related proteins such as hepcidin and ferroportin have been well studied in regards to Ovarian Serous Cystadenocarcinoma, there exists a dearth in analysis of other iron metabolism-related proteins. Given it accounts for a vast majority (>80%) of all ovarian cancers with a poor prognosis, this study filled in the relative gaps of iron-metabolism in regards to ovarian serous cystadenocarcinoma. Using multiple datasets, this study examines the expression and clinical significance of a battery of iron metabolism-related genes in Ovarian Serous Cystadenocarcinoma. Methods Keywords such as "sequestering," "transport," "import" and "homeostasis" were used to query for iron-related gene sets in the MSig database and generate a battery of iron-related genes. One-way ANOVAs with Bonferroni corrections were computed comparing that gene set expression level in TCGA cases of ovarian serous cystadenocarcinoma compared to matched normal TCGA/GTEx data. Further enrichment analysis was conducted to identify what aspects of iron metabolism may be altered in the context of ovarian cancer and non-iron pathways given the wide range of bioactivity in which some of these genes are implicated. Results From a query of 113 genes, 13 genes were shown to be overexpressed in normal cases and 11 in cases of ovarian cancer compared to paired cases after Bonferroni correction ($p < 0.00044$). While enrichment analysis showed changes in biological functions regarding iron metabolism such as ferritin regulation and iron-sulfur cluster binding as hypothesized, interleukin signaling and neutrophil degranulation were also significantly overrepresented ($p < 0.005$). Insulin receptor recycling was highlighted as a function significantly enriched based on protein-protein interaction as well. Conclusions It has been previously established how iron metabolism contributes to tumor aggression and initiation in a variety of cancers, with key players such as hepcidin and its role being examined in ovarian cancer. However, until now there has been limited investigation into how more peripherally related iron-related proteins could be therapeutic targets. Additionally, given the overrepresentation of certain iron metabolism-related genes in possible tumorigenic processes such as neutrophil-mediated matrix remodeling (examples include LCN2 and HMOX1), by targeting genes highlighted in this manner one could observe an effect in many pathways. This offers synergistic approaches to targeting ovarian cancer, which historically has lacked therapeutic advances compared to other kinds of cancers. This in silico approach allows for more targeted wet-lab studies.

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Abstract 2393

Manipulation of cell growth rate alters the rate of total cellular ATP production but not the percent contribution from glycolysis

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Rapid glycolytic flux is frequently associated with proliferative cell growth, both normal and neoplastic. Rapid cell growth, in turn, is asserted to drive a larger proportion of ATP production from glycolytic flux. Although different models exist for how glycolytic ATP production specifically supports rapid cellular proliferation in a way that is distinguishable from oxidative ATP production, there are no direct assessments of the effect of altering cell growth rate on the resulting proportion of glycolytic ATP production.

Study objective: To test the hypothesis that altering cell growth rate directly affects the portioning of ATP production between glycolysis and oxidative phosphorylation.

Methods: Growth rates of cultured C2C12 mouse myoblasts was altered by contact inhibition. ATP production rates were calculated from Seahorse XF measurements of extracellular oxygen consumption and acidification using published methods.

Results: Modulation of cell growth rate was achieved by titrating cell plating conditions, as demonstrated by BrdU incorporation. We demonstrate that decreased cell growth rate slows total cellular ATP production rate by slowing both glycolytic and oxidative ATP production. There was no change in the percentage of ATP production that was glycolytic.

Conclusion: Under the conditions assayed, we do not see evidence that rapid cell growth draws disproportionally more glycolytic than oxidative ATP production. Rather, increases in glycolytic ATP production seen with faster cell growth were attributable to increased ATP demand, that was met by proportional increases in both ATP production pathways.

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Abstract 2406

Glucose oxidation is the preferred metabolic phenotype in proliferating cells

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Proliferating cells often exhibited an elevated rate of glucose fermentation to lactate irrespective of oxygen availability, a phenomenon described as the "Warburg effect." The current thinking in the field is that proliferating cells switched to glucose fermentation, with the implication that fermentation of glucose is preferred during proliferation. Here, by quantifying the activities of mitochondrial pathways and lactate production under various conditions, we show that oxidation of glucose is preferred over fermentation in proliferating cells. We observed that proliferating cells upregulate the flux of transporting NADH into mitochondria and prefer to oxidize glucose-derived NADH by mitochondrial respiration. By contrast, glucose fermentation primarily occurs as a compensatory mechanism when the rate of NADH production from glycolysis exceeds the maximum flux of transporting NADH into mitochondria. Thus, our findings underscore the importance of mitochondria metabolism in proliferating cells and suggest that fermentation, a hallmark of rapid proliferation, is not the preferred metabolic phenotype of proliferating cells.

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Abstract 2434

Mapping Tardigrade Respiration and Evaluating Metabolic Dependence of Osmobiotic Processes

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Tardigrades are semi-microscopic invertebrates known to survive a variety of environmental stressors via a process known as cryptobiosis. Cryptobiosis, a multifaceted biochemical process involving the upregulation of a host of unique protective proteins, enables tardigrades to survive in a stasis-state much longer than their typical lifespan. It has long been reported that upon entering a cryptobiotic state, tardigrades arrest or slow their metabolisms far below that which is required to sustain life normally. Although there exist studies which attempt to elucidate the mechanisms of tardigrade cryptobiosis, few have sought to quantitatively measure the differences in metabolic activity across various stressor-induced responses using modern analytical techniques. Our work focuses on investigating the dependence of tardigrade cryptobiosis induction and maintenance on cellular respiration and metabolism. We have found evidence via electron paramagnetic resonance and biochemical assays that the tardigrade *Hypsibius exemplaris* relies on intracellular ROS generation to enter osmobiosis, the physiochemical state which is formed in response to high concentrations of osmolytes. We have provided further evidence that tardigrades can use the alternative oxidase pathway in lieu of the traditional electron transport chain to undergo osmobiosis. These revelations provide key insight into the metabolic dependence of cryptobiotic processes and further understanding of mechanisms by which tardigrades survive extreme environmental stress.

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Abstract 2456

Distinct metabolic dependencies in pancreatic cancer

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There is current dire need to develop effective therapies for pancreatic ductal adenocarcinoma (PDA), a highly lethal malignancy on the rise with extremely poor prognosis. Although targeting tumor metabolism has been the focus of intense investigation for over a decade now, tumor metabolic plasticity and a high risk of toxicity have challenged this anti-cancer strategy. Here we show using genetic and pharmacological approaches in human and murine *in vitro* and *in vivo* models, that PDA has a distinct dependence on de novo ornithine synthesis (DNS) from glutamine via ornithine aminotransferase (OAT), which supports polyamine synthesis and is required for tumor growth. This directional OAT activity is normally largely restricted to infancy and contrasts with the reliance of most adult normal tissues and other cancer types on arginine-derived ornithine for polyamine synthesis. We find that this dependence associates with arginine depletion in PDA tumor microenvironment, and is driven by mutant KRAS, which induces the expression of OAT and polyamine synthesis enzymes, leading to alterations in the transcriptome and open chromatin landscape in PDA tumor cells. The distinct dependence of PDA but not normal tissue on OAT-mediated DNS provides an attractive therapeutic window for treating pancreatic cancer patients with minimal toxicity.

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Abstract 2466

Selective oxidation of 8-oxo-guanine in double-stranded DNA by an osmium metallointercalator

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Oxidative damage is a causative factor in many diseases, and 8-oxo-guanine (8-oxo-G) is a common oxidation product in DNA that causes missense mutations. Here, we examined whether an osmium metallointercalator, Os(phen)2dppz3+ (phen = 1,10 phenanthroline and dppz = dipyridophenazine) can selectively oxidize 8-oxo-G to form DNA-protein cross-links. With a 3+/2 + couple of 1.15 V, Os(phen)2dppz3+ should be able to oxidize 8-oxo-G (~0.70 V) without oxidizing guanine (~1.30 V). The flash-quench technique has previously been shown to be a simple way to oxidize guanine; in this method, the intercalator Ru(phen)2dppz2+ is excited with blue light, releasing an electron to a quencher, and the Ru(III) form of the intercalator then extracts an electron from guanine. Located in the same column of the periodic table, osmium and ruthenium have similar chemistry, and Os(phen)2dppz3+ is formed photochemically from its Os(II) form in the same fashion as its ruthenium analogue. To produce 8-oxo-G in DNA, the flash-quench technique was employed with photoexcitation and subsequent oxidative quenching of Ru(bpy)32+ (bpy = bipyridine), as this metal complex is readily removed by ultrafiltration. Treated DNA was then mixed with histone protein, Os(phen)2dppz2+ and methyl viologen (MV2+) in a pH 7 phosphate buffer and irradiated for 0–32 minutes with blue laser light to induce DNA-protein cross-linking. Two methods were used to detect the DNA-protein cross-linking. In the chloroform extraction assay, proteinaceous material is extracted into the organic phase, and the amount of cross-link is determined from the 260 nm absorbance of the free DNA remaining. In this uv spectroscopy assay, the amount of cross-linking was much larger for the pretreated DNA containing 8-oxo-G than for untreated DNA. In an analogous gel shift assay experiment using pUC19 DNA, the treated plasmid showed more accumulation of lower mobility material as irradiation times increased. Additionally, a small model system was designed for studying the selective oxidation on a 20-mer DNA duplex. The strand 5′-ATATGATAT8GATATGATAT-3′ (8 = 8-oxo-G) was synthesized with an amino group at the 3′ end, which allowed conjugation to a fluorescent dye, AlexaFluor546. In these gel shift experiments, the fluorescently labeled duplex underwent the flash-quench treatment with Ru(phen)2dppz2+ in the presence of histone, producing a band of intermediate mobility (presumably 1:1 crosslink) and well-shifted material. In contrast, analogous treatment with Os(phen)2dppz2+ produced only the band of intermediate mobility, consistent with the presence of only a single site that is oxidizable by the osmium complex. Lastly, transient absorption spectroscopy was used to determine if the osmium

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complex is capable of oxidizing guanine. By comparing the signals produced for the osmium complex bound to two different 22-mer duplexes, one with all A-T basepairs and the other with a high GC content, it was observed that the transient spectrum is the same for both duplexes, with the spectral characteristics of the Os(III) complex. In contrast, the analogous ruthenium complex, which can oxidize guanine in its Ru(III) form, gives a much different spectrum in the two duplexes. In conclusion, these results show that Os(phen)2dppz3+ is a promising selective oxidant of 8-oxo-guanine in double-stranded DNA.

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Abstract 2467

Investigation of Rambutan and its Molecular Components as Inhibitors of DNA Oxidation

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Oxidative damage is involved in the formation of free radicals, which can cause various diseases. In DNA, the damage is observed primarily at guanine (G) because it is the most easily oxidized base. Rambutan fruit contains a number of antioxidants, being ellagic acid, p-coumaricacid, gallic acid, butylated hydroxytoluene, and corilaginand therefore can lead us to expect inhibition of DNA oxidation. Here, we examined whether an aqueous extract of rambutan flesh and other molecules found in rambutancan inhibit the formation of oxidative DNA-protein crosslinks. The flash-quench technique is a method that is used for guanine oxidation and it can induce DNA-protein crosslinking. In the flash quench technique, the intercalator, Ru(phen)2dppz2+ [phen = phenanthroline, dppz = dipyridophenazine], is excited with a laser and gives an electron to the quencher, Co(NH3)5Cl2+. The oxidized intercalator takes an electron from guanine, creating the guanine radical, which then reacts with protein to form a DNA-protein crosslink. In these experiments, samples containing Ru(phen)2dppz2+, Co(NH3)5Cl2+, histone protein, pUC19 DNA and putative antioxidant solution were irradiated for 0–240 seconds with 442 nm laser light to effect guanine damage. The gel shift assay was used to detect the oxidative DNA-protein crosslinking. In control experiments using pUC19 plasmid as the DNA, there was a significant reduction inintensity for the supercoiled and nicked bands at longer irradiation times, along with the appearance of lowermobility material (crosslinked DNA). In contrast, when the rambutan extract or its molecular components were present, only a small change was observed in the supercoiled and nicked bands, with minimal amounts of lower mobility material. For the molecular components, ellagic acid was seen to exhibit its inhibitory effects at a much lower concentration (~50 micromolar) than did p-coumaric acid, which showed inhibition at 1 mMconcentration. Transient absorption spectroscopy was utilized to monitor the intermediates formed, and a positive signal was observed at 510 nm for a sample containing p-coumaric acid, consistent with formation of a phenolic radical that would result from reaction of guanine radical with the antioxidant. In conclusion, rambutan and its molecular components ellagic acid and p-coumaric acid appear to be effective in inhibiting oxidative damage to DNA, and preliminary evidence points toward the reduction of guanine radical by these molecules. We are currently investigating additional molecules in rambutan, butylated hydroxytoluene (BHT) and gallic acid, to assess their effectiveness at inhibiting guanine oxidation.

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Abstract 2472

Investigation of Cherry Juice and Melatonin as Inhibitors of DNA Oxidation

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Oxidative damage is involved in the formation of free radicals, which can cause various diseases. In DNA, this damage is observed primarily at guanine (G) because it is the most easily oxidized base, and one form of oxidative damage is DNA-protein crosslinking. Here, we examined the effectiveness of cherry juice, as well as melatonin, a component of cherry juice, in preventing oxidative DNA damage. Oxidation was effected by the flash-quench technique, a method used for the selective oxidation of guanine. In the flash quench technique, the photoexcited intercalator, Ru(phen)2dppz2+ [phen = phenanthroline, dppz = dipyridophenazine], gives an electron to the quencher, Co(NH3)5Cl2+. The oxidized intercalator takes an electron from guanine, creating the guanine radical, which then reacts with protein. In our experiment, samples containing Ru(phen)2dppz2+, Co(NH3)5Cl2+, histone protein, pUC19 DNA and water, cherry juice or melatonin (1 mM) were irradiated for 0–5 minutes with blue laser light. In the absence of cherry juice or melatonin, the pUC19 displayed a dramatic intensity loss in the supercoiled band, along with increases in intensity for the nicked band and low-mobility material. In contrast, samples containing cherry juice or melatonin maintained a much higher intensity of supercoiled DNA upon irradiation, and less production of nicked DNA compared to the samples without melatonin. In previous transient absorption spectroscopy experiments with DNA and the peptide Lys-Trp-Lys, guanine radical was seen to decay with the same kinetics as the appearance of the tryptophan radical, which exhibits a positive absorbance near 500 nm. Here, we ran an analogous experiment and found a positive signal at 510 nm for samples with melatonin. Given that both tryptophan and melatonin share the indole group, this positive peak is evidence for the presence of a melatonin radical. In conclusion, cherry juice and melatonin inhibit oxidative damage to DNA, and spectroscopic experiments point to a redox mechanism. In future work, experiments will be carried out to determine a more accurate concentration range for these antioxidative effects of melatonin, a more complete spectral characterization of the signal for melatonin radical. In addition, these experiments are being expanded to other components of cherry juice, such as catechin.

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Abstract 2508

Characterization of HSD17B10 Gene Product involved in Pathogenesis of Neurodegeneration including Alzheimer's Disease

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Xue-Ying He

Type 10 17β-hydroxysteroid dehydrogenase (17β-HSD10) is the HSD17B10 gene product, plays an appreciable role in cognitive functions. This mitochondrial, multifunctional protein is involved in a variety of metabolic pathways including branched-chain amino acid degradation and neurosteroid metabolism to keep allopregnanolone homeostasis. Deacetylation of 17β-HSD10 by Sirtuins can regulate its catalytic activities. It may also act as cardiolipin phospholipase or a member of the Parkin/PINK1 pathway, or bind to cyclophilin D to play a critical role in the control of mitochondrial structure, morphology and dynamics. Furthermore, 17β-HSD10 can serve as an essential component of RNase P necessary for mitochondrial tRNA maturation, or to bind with Aβ thereby enhancing its neurotoxicity to harm brain cells. Even in the absence of Aβ, its quantitative or qualitative variation can also result in neurodegeneration. Since elevated levels of 17β-HSD10 were found in brain cells of Alzheimer's disease (AD) patients and mouse AD models, 17β-HSD10 was considered a key factor in the AD pathogenesis, in addition to Aβ and Tau protein. Nevertheless, data underlying its equivalent :"Aβ-binding-alcohol dehydrogenase (ABAD)" was recently revealed to be unreproducible. Publishing accurate information would certainly encourage researchers to find answers to the question why elevated levels of 17β-HSD10 are present in brains of AD patients as well as mouse AD models. Searching specific inhibitors of 17β-HSD10 may lead to promising candidates to reduce neurodegeneration and open up new approaches for the treatment of AD.

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Abstract 2565

Altered Expression and Activity of Succinate Dehydrogenase in Pancreatic Cancer

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Succinate dehydrogenase (SDH) is an enzyme of the inner mitochondrial membrane that functions in both the tricyclic acid cycle and the electron transport chain. The succinate dehydrogenase enzyme complex and abnormalities associated with it have been linked to a number of different tumor types and malignancies. This link between SDH and tumorigenesis has not, however, been specifically linked to pancreatic cancer. This study looks at the gene and protein expression of SDH subunits in pancreatic cancer cells in comparison to normal pancreatic cells using RT-PCR and Western blotting. Additionally, the mitochondrial fraction from pancreatic cells was isolated and analyzed for SDH enzymatic activity. Metabolism of cells was also measured by resazurin reduction in the presence and absence of succinate. The results demonstrate an altered gene and protein expression, accompanied by an increase in SDH activity in pancreatic cancer cells compared to a noncancerous human pancreatic cells that correlated to an increase in metabolism in the cells when fed succinate. To conclude, the data highlights a different metabolic profile for pancreatic cancer. These differences in expression and activity of SDH may help elucidate a mechanism of tumorigenesis in pancreatic cancer and will guide future research to evaluate the link between SDH and pancreatic cancer.

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Abstract 2575

Hydrogen sulfide induces long-term changes in mitochondrial energy metabolism

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Since its discovery as a gaseous signaling molecule, an array of physiological effects has been associated with hydrogen sulfide (H2S) in the cardiovascular, nervous, and gastrointestinal systems. Cells maintain low steady-state levels of H2S, a well-known respiratory toxin, and prevent its accumulation via clearance by the mitochondrial sulfide oxidation pathway to thiosulfate and sulfate. The first step in the sulfide oxidation pathway is catalyzed by sulfide quinone oxidoreductase, a flavoprotein reductase that resides in the inner mitochondrial membrane. Low concentrations of sulfide are rapidly cleared by this pathway, but high concentrations of H2S inhibit complex IV in the mitochondrial electron transport chain (ETC). While 10–80 nM intracellular H2S is typical in various tissues, 0.2–2.4 mM H2S has been detected in human gut, which hosts a variety of H2S-producing bacteria. Hence, colonic epithelial cells are routinely exposed to bacteria derived H2S. The hydrophobicity of H2S allows its free movement across membranes, increasing colonic exposure to the gas. Accordingly, colonocytes are adapted to survive acute exposure to high H2S levels. Even so, cells in culture are not well adapted to handling more than one exposure to high sulfide (100 μM), which induces long-lived changes in mitochondrial bioenergetics. We refer to this phenomenon as "H2S memory," and hypothesize that it results from a reductive shift and/or an increase in local O2, resulting from complex IV inhibition that propagates through mitochondrial metabolism, affecting function. We have characterized these long-term effects of H2S on mitochondrial bioenergetics utilizing oxygen consumption assays and determined genetic and chemical factors that modulate H2S memory. We find that acute sulfide treatment rapidly induces H2S memory and lipid droplet accumulation and causes long lived changes in mitochondrial morphology and cristae structure.

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Abstract 2578

Tumor suppressor p53 promotes ferroptosis in oxidative stress conditions independent of modulation of ferroptosis by p21, CDKs, RB, and E2F

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Samkeliso Dlamini, Viranga Tillekeratne, William Taylor

p53 is a well-established critical cell cycle regulator. By inducing transcription of the gene encoding p21, p53 inhibits cyclin-dependent kinase (CDK)-mediated phosphorylation of cell cycle inhibitor retinoblastoma (RB) proteins. Phosphorylation of RB releases E2F transcription factor proteins that transactivate cell cycle–promoting genes. Here, we sought to uncover the contribution of p53, p21, CDK, RB, and E2F to the regulation of ferroptosis, an oxidative form of cell death. Our studies have uncovered unexpected complexity in this regulation. First, we showed that elevated levels of p53 enhance ferroptosis in multiple inducible and isogenic systems. On the other hand, we found that p21 suppresses ferroptosis. Elevation of CDK activity also suppressed ferroptosis under conditions where p21 suppressed ferroptosis, suggesting that the impact of p21 must extend beyond CDK inhibition. Furthermore, we showed that overexpression of E2F suppresses ferroptosis in part via a p21-dependent mechanism, consistent with reports that this transcription factor can induce transcription of p21. Finally, deletion of RB genes enhanced ferroptosis. Taken together, these results show that signals affecting ferroptotic sensitivity emanate from multiple points within the p53 tumor suppressor pathway.

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Abstract 2601

A redox cycle with complex II prioritizes sulfide quinone oxidoreductase-dependent H2S oxidation

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H2S is a signaling molecule that is produced by the transsulfuration pathway enzymes, cystathionine b-synthase (CBS) and g-cystathionase (CTH) or via gut microbial metabolism. Sulfide quinone oxidoreductase (SQOR) catalyzes the first step in the mitochondrial H2S oxidation pathway, which utilizes CoQ as an electron acceptor and connects to the electron transport chain at the level of complex III. We have discovered that at high H2S concentrations, which inhibit complex IV and blocks forward electron flow, a new redox cycle is established between SQOR and complex II, operating in reverse with fumarate serving as a terminal electron acceptor. We have identified that SQOR deficiency in endothelial cells limits cell proliferation under hypoxia. Dissipation of the mitochondrial NADH pool rescues SQOR-induced hypoxic growth restriction. Tumor xenografts on whole-body WBCreSQORfl/fl mice exhibit lower mass and reduced angiogenesis compared to SQORfl/fl controls. Femoral artery ligation revealed reduced muscle angiogenesis in WBCreSQORfl/fl mice. Our study nominates SQOR inhibition as a metabolic vulnerability for endothelial cell proliferation and neovascularization.

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Abstract 2609

'Fix'-ing to understand electron flow in a purple nonsulfur bacterium

Kathryn Fixen, *University of Minnesota*

Bacteria are promising biocatalysts for the production of biofuels and bioproducts because they can tap into sources of energy that we are still struggling to use (e.g. plant biomass, sunlight, etc.). The ATP and electrons generated from metabolizing these sources can power metabolic pathways that produce energy-rich compounds. Anaerobic bacteria and archaea have evolved diverse ways of managing electron flow to pathways that often naturally result in the release of compounds like butanol, ethanol, methane, hydrogen, etc. Understanding mechanisms that control electron flow is necessary to get these organisms to produce more of these compounds. In my lab, we are interested in electron transfer pathways that involve the protein electron carrier known as ferredoxin (Fd). Fds provide reducing power for enzymes that can carry out some of the most difficult reduction reactions on the planet (e.g. biological nitrogen fixation) and are ideal targets to control electron flow because Fds have properties that can, in theory, be manipulated to form new interactions to re-route electron flow. However, this remains challenging because for many Fds these properties are ill-defined. In this talk, I will describe how we evolved a new electron transfer pathway to nitrogenase, which we believe will provide insight into how interactions between Fds and their partner proteins control electron flow.

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Abstract 2614

Mechanistic studies of RquA-mediated conversion of ubiquinone derivatives to rhodoquinone using *in vitro* assays

Kristian Mankiller, *Gonzaga University*

Sophia Atin, David Langelaan, Jennifer Shepherd

Ubiquinone (UQ) is a prenylated 1,4-benzoquinone which is used as an electron carrier in the electron transport chain (ETC) for aerobic metabolism. Rhodoquinone (RQ), which has an amino group replacing the 5-methoxy group of UQ, is an essential electron carrier used in anaerobic metabolism. The RquA enzyme can interconvert UQ substrates into RQ products in the presence of S-adenosyl-L-methionine (SAM) and manganese (II) *in vitro*. The RquA-mediated pathway from UQ to RQ provides a potential anti-microbial drug target. Anaerobic metabolism is critical for microbial organism survival inside of a host, so disruption of the ETC through the development of novel treatments that target this mechanism could prove vital in the treatment of microbial infections. In order to further study the RquA mechanism, the 5-methoxy substituent of UQ was modified, in addition to the length and functional groups of the prenyl tail, to yield UQ derivatives. The altered 5-methoxy derivatives synthesized were ethoxy-UQ3, propoxy-UQ3, and butoxy-UQ3. The control UQ derivative, UQ3, was previously synthesized. Commercially available derivatives with modified prenyl tails were UQ0, UQ1, UQ4, and decyl UQ. MitoQ15 and MitoQ15-OH, two other UQ derivatives with functionalized tails, were synthesized from UQ0. The various UQ derivatives were tested using *in vitro* RquA assays to better understand the RquA-mediated mechanism for synthesis of RQ. Quantitation was provided for each assay by LC-MS. Parameters for modifications of UQ substrates for which functionality could be retained were found.

This work was funded by a New Frontiers in Research Fund—Exploration Grant (NFRFE-2018-01643) and the O'Leary Scholar Award (Gonzaga University). Student stipend support was provided by a grant from the Howard Hughes Medical Institute through the Undergraduate Science Education Program.

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Abstract 2617

Efficacy of Triamcinolone Acetonide to Reduce Pain in Patient with Knee Osteoarthritis: a Case Report

Hanik Hidayati, *Airlangga University*

Ricky Wicaksono, Siti Khaerunnisa, Lynda Hariani

Introduction: Knee osteoarthritis (KOA) is a chronic debilitating disease caused by the deterioration of joint cartilage due to repeated friction causing stiffness, pain, and limited movement of the knee joint. The chronicity and progression of KOA is the second main cause of socio-economic burden and disability worldwide.

Case report: A female, 81 years old, complaining of right knee pain with a VAS of 8 since last year. The pain gets worse when walking, stair-climbing, and weightlifting. She complained of stiffness in her knees every morning when she woke up, lasting for about 20 minutes but improved with activity. X-ray examination of the knee showed Kellgren-Lawrence grade 3 osteoarthritis. She was given triamcinolone acetonide (TAC) injection with a superolateral approach, and 1 week's evaluation showed improvement of VAS to 3.

Discussion: KOA is a degenerative disease due to the "wear and tear" process in the knee joint. The risk factors for KOA are multifactorial, including obesity, trauma, repetitive knee joint activity, older age, genetics, and female gender. The OA process begins with chronic wear and tear resulting in macro and micro trauma that changes the composition of the joints and cartilage. Biochemical processes of cartilage, subchondral bone, and synovium lead to cartilage breakdown, osteophyte formation, and subchondral bone sclerotic and synovial changes. Repeated injury of chondrocyte cells stimulates the generation of pro-inflammatory cytokines such as interleukin-1, interleukin-6, and TNFα which result in chronic inflammation of cartilage tissue. This process results in symptoms such as stiffness, pain, and limited movement of the knee joint. Treatment of KOA aims to reduce pain, optimize function, reduce physical activity limitations, inhibit progression, and prevent complications. Intraarticular injection using corticosteroid has anti-inflammatory and immune suppressant effects with complex and diverse mechanisms, and it works by entering immune cells and binding to steroid receptors in the cell nucleus and inhibiting various gene cascades in cells. Corticosteroids can lessen vascular permeability, phagocytes and accumulation of immune cells, and inflammation by inhibiting the production of metalloproteases, neutrophil superoxide, and several inflammatory mediators such as leukotrienes and prostaglandins. Administration of corticosteroids will result in the improvement of inflammatory responses and reduce redness, heat, swelling, and pain. The patient in this report experienced an improvement in her VAS from 8 to 3 after the TAC injection.

Conclusion: KOA is a degenerative disease that causes socio-economic burden and disability. The target of treatment is to reduce pain, optimize joint motion function, reduce

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physical activity limitations, inhibit disease progression, and prevent complications. TAC injections have been shown to improve the pain scale in this case report.

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Abstract 2618

The Combination Multilayer Inner Absorbable Suture Materials Polydioxanone As Management In Keloid Scar: A Case Report

Lynda Hariani, *Universitas Airlangga*

Siti Khaerunnisa, Hanik Hidayati

Introduction: Mature and massive keloids usually need a surgical intervention combined with low-dose radiotherapy to reduce recurrence rate. Therefore, keloid management is considered as a problem for plastic surgeon.

Case report: A 62 years old male came with a recurrent mass on his chest. It occurred after his previous surgery 1 year ago which was thoracotomy. After 2 weeks, the scar rises with a flat surface and itchy sensation. It occurred around 5 cm at the beginning, then the patient had surgical procedure with local anesthesia. However, the mass started to reoccur and bigger than before. Keloid excision was done to the patient, with the depth of fascia and undermining area of the scar (± 3 – 4 cm) until the skin can be near enough. Multilayer suturing using polydiaxanone (PDS) has been used to reduce tensile strength. During his last visit, the scar was healed, no dehiscence found, the skin looks relaxed and no raising scar to be found.

Discussion: During wound healing process in remodeling phase, a dense package of collagen was formed which lead to wound closure and resulting a mature scar. Multilayer suturing can maintain the tensile strength and reduce the mechanical load factor in the surgical wound. Furthermore, prolonged presence of suture material to avoid excessive tension on the dermal layers may benefits wound healing process. Absorbable suture materials such as polydiaxanone (PDS), are synthetic absorbable sutures that have been found to be nonantigenic and nonpyrogenic and only cause a mild tissue reaction during resorption. In addition, polydioxanone (PDS) was developed to provide wound support for an extended period of time.

Conclusion: Various methods keloid management are currently available and emerging therapies are also being studied. This case report shows a multilayer inner absorbable suture with suitable choice of suture material such us polydiaxanone (PDS) can be an option in treating problematic and disfiguring keloid.

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Abstract 2640

Targeting a Novel PAK1 Signaling Pathway Required for Skeletal Muscle Mitochondrial Function Towards Reversing Insulin Resistance

Rekha Balakrishnan, *City of Hope*

Debbie Thurmond

Skeletal muscle is a major regulator of peripheral insulin sensitivity. We previously showed that p21-activated kinase (PAK1) is essential for skeletal muscle insulin sensitivity, via a role in actin remodeling to facilitate glucose uptake. However, our transcriptomic analysis of skeletal muscle-specific inducible knockout of PAK1 (SkmPAK1-iKO) in mice revealed high levels of mitophagy markers. Thus, our objective was to define the role of PAK1 in skeletal muscle mitochondrial function, and potential implications for insulin resistance. We now present evidence that PAK1 plays a previously unknown and essential role in skeletal muscle mitochondria: PAK1 knockdown using siRNA in L6 myotubes impaired mitochondrial-associated genes and proteins, decreased mitochondrial copy number, impaired mitochondrial structure, and reduced mitochondrial respiration. Conversely, adenovirus-mediated PAK1 restoration in insulin-resistant L6 myotubes increased mitochondrial copy number, respiration, reduced superoxide radicals and preserved mitochondrial structure. The expression of PGC-1a and genes involved in oxidative phosphorylation were previously found to be decreased in muscle from individuals with type 2 diabetes and impaired glucose tolerance; PAK1 knockdown in skeletal myotubes (rat L6) similarly decreased expression of PGC-1a. Toward understanding the linkage between PAK1 signaling and PGC-1a gene expression, we investigated PAK1 activation, localization, and candidate signaling elements using cultured myotubes. Activated PAK1 was detected exclusively in non-nuclear fractions, suggesting that PAK1 does not directly induce nuclear-localized gene expression changes. Further, inhibition of PAK1 activation, using the pharmacological inhibitor IPA3, significantly decreased the abundances of proteins known to transcriptionally regulate PGC-1a, namely p38 mitogen-activated protein kinase (p38MAPK) and activating transcription factor 2 (ATF2) in L6 myotubes. While inhibition of p38MAPK activity (using SB202190) reduced PGC-1a gene expression, overexpression of PAK1 in SB202190 treated cells could not rescue PGC-1a levels to normal, suggesting that PAK1-induced PGC-1a expression is mediated via p38MAPK. Further, IPA3-treated L6 myotubes exhibited decreased phosphorylated ATF2 (downstream transcription factor activated by p38MAPK) levels in the nuclear fraction. Concomitantly, PAK1 enrichment in insulin resistant L6 myotubes showed increased expression of pATF2 compared to insulin resistant control cells; further studies will evaluate the nuclear localization of pATF2 in PAK1 enriched cells. Overall, our results reveal that PAK1 is essential for muscle mitochondrial function, and it is mediated through a p38MAPK → ATF2 → PGC-1a signaling pathway, providing a new signaling cascade for potential interventional strategies for skeletal muscle insulin resistance in pre- and type 2 diabetes.

This work is generously supported by grants from the National Institute of Diabetes and Digestive Kidney Diseases (DK067912, DK112917, and DK102233) to D.C.T., and an American Heart Association Postdoctoral fellowship (#19POST905770) to R.B.

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Abstract 2651

Complementation of *Escherichia coli* Menaquinone Biosynthetic Mutants by Novel Plant Phylloquinone Biosynthetic Genes Supports a Noncanonical Phylloquinone Pathway at the Plasma Membrane

Mya Covington, *University of Georgia*

Ing-Gin Chen, Chung-Jui Tsai

Phylloquinone (vitamin K1) is an essential electron acceptor in chloroplast for photosynthesis. The canonical pathway contains two terminal chloroplast-targeting genes: MenA1 and MenG1. Recently, MenA2 and MenG2 genes lacking chloroplast-targeting sequences, were identified in the obligate parasitic plant *Phelipanche aegyptiaca*. The MenA2/G2 genes were confirmed to target the plasma membrane. This noncanonical vitamin K1 pathway could possibly support nonphotosynthetic parasitic plants during their life cycles and studying the pathway can improve our understanding of parasitic plant infections in important agricultural crops. *Rehmannia glutinosa*, a photosynthetic medicinal Chinese herb, carries both MenA2/G2 and the canonical MenA1/G1 suggesting that the phylloquinone pathway evolved to exist in two subcellular compartments. *Escherichia coli* was used as a model system to investigate the functionality of *R. glutinosa* and *P. aegyptiaca* MenG2 and MenA2 genes due to the presence of an analogous menaquinone (vitamin K2) biosynthetic pathway. The MenG2 gene of *R. glutinosa* can complement strain JW5581 *E. coli* (ΔmenG) in vitamin K2 production. This was verified first with sequencing prior to transformation and after a colorimetric assay. *R. glutinosa* MenG2 complementation was observed phenotypically in a selenate assay requiring functional MenG and MenA genes to reduce selenate to elemental red selenium. Complementation of *R. glutinosa* MenG2 within *E. coli* synthesizing vitamin K2 for plasma membrane electron transport further supports the hypothesis of a noncanonical vitamin K1 pathway. However, expression of either bacterial MenA or plant-derived MenA2 genes in strain AN67 *E. coli* (ΔmenA) appeared to be lethal, resulting in frequent mutations of the inserted genes, rendering them non-functional. As menaquinone mainly functions in microaerobic or anaerobic conditions, MenA gene overexpression under aerobic conditions might have been detrimental to bacterial growth. Further experiments are necessary to optimize permissible conditions for functional complementation of ΔmenA *E. coli*.

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Abstract 2655

Roles of ENPP1 Activity and 2′3′-cGAMP Degradation in Ovarian Cancer Cell Lines

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Hannah Briggs, Margaret Collins, Lauren Clements, Olivia Haskin, Jiajia Ji, Phillip Rzeczycki, David Taylor

The American Cancer Society estimates that approximately 20,000 women will be diagnosed with ovarian cancer, and nearly 13,000 more are expected to die from this disease in the United States this year. Ovarian cancer is a complex and heterogeneous disease with various subtypes and diverse molecular mechanisms of pathogenesis. Among different forms, epithelial ovarian cancer is the most common, representing up to 90% of total reported cases. Due to the lack of early diagnostic tools, the prognosis is mainly poor, as most cases are typically identified in advanced stages. Thus, a more thorough understanding of the inherent complexities of the disease, in terms of its biochemical etiology, will allow for identification of specific molecular and genetic profiles that could lead to more effective treatment plans. Recent studies have shown that ENPP1 may play a role in cancer cell proliferation, migration, and invasion in ovarian and other types of cancer. ENPP1 is a glycosylated type II transmembrane protein acting as a pyrophosphatase and phosphodiesterase with broad specificity. ENPP1's substrates include mononucleotides and cyclic dinucleotides such as 2′3′-cGAMP. In this study, we investigated whether ENPP1 and its hydrolysis of 2′3′-cGAMP can affect disease progression in ovarian cancer using cell lines such as CaOV-3, SK-OV-3, and PA-1. We tested whether the supplementation of 2′3′-cGAMP to induce the production of type I interferons, coupled with the inhibition of ENPP1, can affect the proliferation of these cell lines. To this end, we used a fluorescence cell-based assay to directly monitor the activity of ENPP1, and used established 2′,3′-cGAMP ELISA and TR-FRET assays to monitor the degradation of 2′3′-cGAMP in the presence and absence of ENPP1 inhibitors. ENPP1 was inhibited using compounds such as ENPP1 Inhibitor C, Inhibitor 43, or Inhibitor 4e. The viabilities of different ovarian cancer cell lines were evaluated by monitoring cellular metabolic activity and cell cycle progression. Our results show that cell proliferation was reduced in all ovarian cell lines studied upon inhibition of ENPP1, suggesting that ENPP1 is a potential therapeutic target in epithelial ovarian cancer and could be used as a potential marker for disease status.

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Abstract 2724

Genes of Alcohol Metabolism and Cancer Patient Survival Analysis

Dabin Jeong, *Lawrence University*

Kimberly Dickson

Alcohol metabolism mainly occurs in the liver by metabolic enzymes that catalyze ethanol oxidation. According to epidemiological studies, alcohol consumption has been associated with a risk for esophageal, breast, and liver cancer. The objective of this study is to study the correlations of the expression level of alcohol metabolism genes with 33 different cancer types using the TCGA data set. Patient data stored in TCGA was used for the analysis of survival correlations and differential gene expressions between normal and tumor types. Using TCGA data from Gene Expression Profiling Interactive Analysis 2 (GEPIA2), we performed survival analysis of different alcohol metabolism genes in 33 different types of tumors. We found significant correlations between genes known for alcohol metabolism with a variety of different types of cancers. Specifically, four alcohol dehydrogenase (ADH) genes were found to be correlated with the survival of patients with liver hepatocyte carcinoma (LIHC). We also found that the high catalase (CAT) expression group was significantly associated with the unfavorable outcome of adrenocortical carcinoma (ACC). Interestingly, low CAT expression was associated with the poor outcome of kidney renal clear cell carcinoma (KIRC). We propose the insight that the multiple different alcohol metabolism genes were correlated with the patient survival of the different types of cancer.

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Abstract 2737

Stress-induced mitochondrial remodeling and metabolic resilience

Deborah Muoio, *Duke University*

Studies using mass spectrometry-based metabolomics have produced substantial evidence linking states of cardiometabolic stress to elevated levels of mitochondrial-derived acylcarnitine (AC) metabolites in tissues and blood. Most ACs derive from acyl-CoA intermediates of mitochondrial fatty acid catabolism, and dysregulated lipid oxidation is a hallmark of pre-diabetes, cardiac dysfunction and exercise intolerance. For the past decade, we have remained keenly committed to answering a crucial question: What is this metabolite signature telling us about the interplay between mitochondrial stress and metabolic health? Whereas powerful multi-omics molecular profiling technologies can lend clues, they do not tell us how well (or not) mitochondria resident in specific tissues are able to maintain the free energy of ATP hydrolysis (ΔGATP, i.e. ATP:ADP ratio)– which is the energetic currency that powers normal cell and organ function. We came to recognize that progress toward deciphering the precise role of mitochondrial- derived ACs as markers and/or mediators of disease requires new approaches to assess carbon flux and bioenergetics in intact mitochondria working to maintain ΔGATP in the context of physiologically relevant energy demands and thermodynamic constraints (i.e., backpressure). To this end, we developed a multiplexed assay platform for deep and comprehensive phenotyping of respiratory kinetics and thermodynamics under multiple substrate and energetic conditions. We have been combining this platform with MS-based proteomics, metabolomics and 13C metabolic flux analysis to understand how mitochondria respond and adapt to a wide variety of physiological and pathophysiological settings of nutrient and/or energy stress (e.g. obesity, heart failure, exercise training and time-restricted feeding). This seminar will highlight emerging insights into functional relevance of AC accumulation and the role of mitochondrial ketone flux in defending energy stability and promoting metabolic resilience.

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Abstract 2738

Mitochondrial Dynamics and Cardiometabolic Disease

E. Dale Abel, *UCLA*

Mitochondria are dynamic organelles undergoing repeated cycles of fusion and fission. This fundamental process is largely believed to play essential roles in mitochondrial quality control, mitochondrial biogenesis and cellular homeostasis. Mitochondrial dynamics is regulated by a conserved family of dynamin-related GTPAses such as OPA1 and mitofusins. Perturbations in mitochondria dynamics are being recognized as playing a role in the pathophysiology of metabolic disease. The presentation will summarize work linking altered regulation of mitochondrial dynamics with the activation of mitochondrial stress pathways that regulate systemic metabolic homeostasis and pathophysiologic pathways that exacerbate cardiovascular complications of obesity and diabetes. For example, OPA1 cleavage is required for brown adipose tissue (BAT) activation and perturbations in OPA1 function impact BAT activation. Impaired mitochondrial dynamics activate stress pathways leading to the secretion of circulating factors such as fibroblast growth factor 21 and GDF15 that have significant effects on systemic metabolic homeostasis, energy expenditure and body weight.

NIH, AHA

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Abstract 2740

De novo NADP+ synthesis powers breast cancer metastatic outgrowth

Ana Gomes, *H. Lee Moffitt Cancer Center*

Metabolic reprogramming and metabolic plasticity allow cancer cells to fine-tune their metabolism and adapt to the ever-changing environments of the metastatic cascade, for which lipid metabolism and oxidative stress are of particular importance. Continuous production of NADPH, a cornerstone of both lipid and redox homeostasis, is essential for proliferation and cell survival suggesting that cancer cells may require larger pools of NADPH to efficiently metastasize. NADPH is recycled through reduction of NADP+ by several enzymatic systems in cells; however, de novo NADP+ is synthesized only through one known enzymatic reaction, catalysed by NAD+ kinase (NADK). Here, we show that NADK is upregulated in metastatic breast cancer cells enabling de novo production of NADP(H) and the expansion of the NADP(H) pools thereby increasing the ability of these cells to adapt to the oxidative challenges of the metastatic cascade and efficiently metastasize. Mechanistically, we found that metastatic signals lead to a histone H3.3 variant-mediated epigenetic regulation of the NADK promoter, resulting in increased NADK levels in cells with metastatic ability. Together, our work presents a previously uncharacterized role for NADK and de novo NADP(H) production as an important contributor to breast cancer progression and suggests that NADK constitutes an important and much needed therapeutic target for metastatic breast cancers.

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Topic Category Organelles and Compartments

Abstract 148

Regulation of inter-organelle contact formation by phosphorylation

Catherine Tomasetto, *University of Strasbourg*

The formation of contacts joining distinct organelles requires in many cases protein-protein interaction. On the endoplasmic reticulum (ER), members of the Vamp Associated Protein (VAP) family are often implicated in contacts. VAP-A, VAP-B and MOSPD2, share a Motile Sperm Domain (MSP) which binds FFAT (two phenyl alanine in an acidic tract) motifs. Interaction between the MSP domain and a FFAT motif forms a stable protein complex. Live imaging however shows that inter-organelles contacts are dynamic structures: organelles can attach and detach overtime, indicating that association and dissociation mechanisms exist. The primary sequence of the FFAT motif of the StAR Related Lipid Transfer Domain Containing 3 (STARD3) protein gave insights into a potential mechanism. We identified in STARD3 a serine amino acid (aa) instead of an acidic aa at a key position of the FFAT motif. This finding let us to show that phosphorylation at a serine residue can substitute for the acidic aa. We then defined a new kind of FFAT motif called phospho-FFAT. By scanning the human proteome, we noted that Phospho-FFAT were as frequent as conventional FFAT motifs. This work supports the idea that phosphorylation and dephosphorylation can act as a switch and provides a molecular mechanism explaining the plasticity of some inter-organelle contacts.

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Abstract 150

A widespread encapsulin-based elemental sulfur storage system in bacteria?

Robert Benisch, *University of Michigan-Ann Arbor*

Michael Andreas, Tao Ma, Nancy Muyanja, Tobias Giessen

Encapsulins are microbial protein compartments that specifically sequester dedicated cargo enzymes to spatially confine enzymatic reactions, store nutrients, and control reactivity. Here, we report on a recently discovered encapsulin system with cysteine desulfurase (CD) cargo able to accumulate elemental sulfur in crystalline form, which may represent a novel sulfur storage or distribution system in bacteria. Sulfur deposits inside encapsulin shells were initially observed in cryo-electron microscopy (cryo-EM) micrographs of purified heterologously expressed CD-loaded encapsulins as electron dense puncta. Using high-resolution transmission electron microscopy (HR-TEM) and energy-dispersive X-ray spectroscopy (EDS), we determined that these puncta represent zero-valent sulfur crystals. To better understand how encapsulation influences CD catalysis, we performed *in vitro* desulfurase assays which showed that encapsulation substantially increases CD catalytic activity in the absence of a sulfur acceptor. S(0) is generally not stable in the presence of reducing agents, being quickly reduced to S(2-). We hypothesized that the encapsulin shell may prevent S(0) reduction by acting as a physical barrier for cellular reducing agents. Thus, to investigate if the observed S(0) crystals could be stable under the reducing conditions of the bacterial cytosol, we exposed purified CD encapsulins containing S(0) crystals to biologically relevant reducing conditions. Colorimetric determination of S(0) after reducing agent exposure confirms that S(0) persists when challenged with up to 20 mM L-glutathione or clarified bacterial lysate. Together our data show that encapsulating CD in a protein shell leads to (i) increased CD catalytic activity, potentially by molecular crowding of persulfide intermediates, (ii) the formation of crystalline elemental sulfur, potentially induced by the nano-scale accumulation of persulfide chains, and (iii) the protection of crystalline S(0) from physiologically relevant reducing agents, indicating that these sulfur crystals could exist in the bacterial cytosol. Together, our data suggest that CD encapsulins may represent an unprecedented mode of sulfur storage widespread in bacteria.

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Abstract 1344

Multi-omics investigation of peroxisomal quality control

DurreShahwar Muhammad, *Rice University*

Natalie Clark, Elizabeth Chatt, Richard Vierstra, Bonnie Bartel

Peroxisomes are multifunctional membrane-bound organelles found in most eukaryotes that sequester essential metabolic reactions. In plants, peroxisomes house fatty acid β-oxidation, the glyoxylate cycle, aspects of photorespiration, and synthesis of some hormones. Many of these vital reactions generate damaging reactive oxygen species (ROS) that also function as signaling molecules. Peroxisomes also contain antioxidant enzymes to decompose these ROS. During plant development, or when antioxidant systems are inadequate, protein damage ensues, requiring additional quality control mechanisms. Peroxisomes house chaperones and proteases, including LON2 (which has both chaperone and proteolytic activity), that can refold or dispose of individual proteins, whereas obsolete and damaged peroxisomes can be degraded through pexophagy, a specialized form of autophagy. Despite the importance of peroxisomes and their interactions with other key organelles, our understanding of the mechanisms that trigger or prevent plant peroxisome turnover is minimal. We are leveraging the recent finding that functional peroxisomes are targeted for destruction by an overzealous autophagy machinery when the Arabidopsis LON2 protease is dysfunctional by elucidating the LON2 function(s) that normally protect Arabidopsis peroxisomes from pexophagy. As a first step towards identifying LON2 substrates, we used proteomic analysis to compare proteins that accumulate when LON2 is dysfunctional in the presence and absence of autophagy. We are coupling our proteomics data with transcriptomic analysis of the same genotypes to unravel transcriptional and post-transcriptional responses. Analysis of this multi-omics landscape will reveal not only LON2 substrates but also the cellular signaling responses that ensue when pexophagy is heightened or prevented.

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Abstract 1351

Yes! Bacteria have organelles! How are they organized?

Anthony Vecchiarelli, *University of Michigan*

Bacteria have a diversity of organelles involved in essentially all aspects of cell function. Yet, the mechanisms governing organelle assembly, organization, and homeostasis remain largely unstudied in bacteria. The cytoskeleton and motor proteins are well known for organizing the membrane-bound organelles of eukaryotes. But bacteria lack extensive membrane-bound organelles, cytoskeletal structures, and linear motors. Instead, bacterial organelles are largely protein-based and a widespread family of proteins, called ParA/MinD (A/D) ATPases, are responsible for their subcellular organization, but the mechanisms remain unclear. My lab is focused on the molecular mechanisms underlying the subcellular organization of bacterial organelles, of which I will discuss 3 research vignettes. (1) Our focus so far has been on a carbon-fixing icosahedral organelle called the carboxysome. Carboxysomes encapsulate the most abundant enzyme on Earth, Rubisco, within a selectively permeable protein shell to create the high CO2 environment needed for efficient CO2 fixation. Carboxysomes are responsible for almost half of global CO2 fixation, and are therefore of great ecological and biotechnological interest; especially in the face of our climate crisis. The bulk of our work has been centered on our discovery of the carboxysome positioning system. (2) While performing this work, several recent studies found that carboxysomes and several other bacterial organelles behave as liquids. The carboxysome, and its positioning system we identified, represent a tractable model for understanding the spatial regulation of liquid-like organelles. We are interested in determining the role of material state in organelle assembly, function, and organization in the cell. (3) Finally, the list of A/D ATPases that spatially organize bacterial organelles is growing. We found that a third of sequenced bacteria encode multiple A/D ATPases, each of which positions a specific cellular cargo. Yet, how A/D ATPases coexist and function in the same cell has never been studied. The final project I will discuss focuses on understanding how organelle trafficking is coordinated in the cell. Overall, our work thus far has uncovered unknown, unique molecular mechanisms of positioning and confinement in the cell.

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Abstract 1355

Performing mechanistic cell biology with condensate reconstitution assays

Simon Alberti, *TU Dresden*

Biomolecular condensates formed by phase separation are membraneless compartments in the cytoplasm and nucleoplasm of cells, which have major roles in cellular organization and physiology. RNP granules are a specific type of condensate that assemble from RNA-binding proteins and RNA. In this talk, I will discuss how the concept of biomolecular condensates has expanded our view of RNP granules and their link to disease, aging and the cellular stress response. I will introduce *in vitro* reconstitution systems based on the concept of phase separation that now allow us to reconstruct RNP granules in the test tube. Using these reconstitution systems as well as innovative imaging approaches and biophysics, we have gained important insights into the molecular rules of RNP granule assembly, such as the driving forces and amino acids that govern condensation, the conformational changes underlying assembly and molecular mechanisms of condensate regulation and control. I will further discuss how the concept of phase separation has allowed us to dissect the functions of RNP granules, and I will demonstrate how condensate formation is used by cells to sense and respond to changes in the environment and regulate fundamental cellular processes such as protein synthesis.

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Abstract 1459

Emergent properties of heterochromatin component condensates

Priyasha Deshpande, *CUNY Graduate School and University Center*

Patrizia Casaccia, Shana Elbaum

Biomolecular phase separation has revolutionized the field of cell biology, with much interest directed towards unraveling the role of condensate formation in nuclear organization. The Heterochromatin protein 1alpha (HP1), encoded by the gene Cbx5, binds specifically to the repressive H3K9me3 mark on histone H3 via its chromodomain, but does not bind to other methylated lysine marks, such as H3K4me3 (associated with a transcriptionally active state). It has been recently reported that phase separation of HP1a with DNA, in part, contributes to the formation of condensed, mechanically stable chromatin states. However, whether its ability to recognize H3K9me3 marks play an active role in modulating its material properties and dictating emergent functions of condensates needs to be investigated. While heterochromatic domains are mostly organized at the nuclear periphery and related to silent gene expression, it is becoming clear that heterochromatin in gene-poor regions also serves as mechanosensor, with the ability to undergo rapid disassembly and spread across the nuclear space in response to mechanical stress and other cellular cues. These contrasting characteristics raise several questions regarding the material properties of HP1a-driven heterochromatin condensates. Even though HP1a-DNA condensates are well characterized, the role of repressive H3K9me3 mark in dictating the emergent material state of heterochromatin condensates is poorly understood. In this study, we leverage a reductionist system comprised of histone H3-tail, heterochromatin protein 1a (HP1a) and DNA to examine the role of the repressive H3K9me3 mark in contributing to the viscoelasticity of HP1a-DNA assemblies. Combining confocal fluorescence microscopy and rheological approaches, we demonstrate that unmodified H3 compared to H3K9me3 tails give rise to condensates with markedly different dynamics and material properties. Remarkably, H3K4me3 tails, which are associated with transcriptionally active chromatin, resemble that of unmodified H3 tail –indicating that trimethylation alone is not responsible for the observed differences. Taken together, our results implicate a direct role for epigenetic modifications in the regulation of material properties of phase separated chromatin bodies.

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Abstract 1462

Structure, biogenesis, and engineering of the pyrenoid

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Jessica Hennacy, Nicki Atkinson, Shan He, Moritz Meyer, Elizabeth Freeman-Rosenzweig, Howard Griffiths, Luke Mackinder, Alistair McCormick

Approximately one-third of global CO2 fixation occurs in an overlooked algal organelle called the pyrenoid. The pyrenoid enhances CO2 fixation by supplying the CO2-fixing enzyme Rubisco with a high concentration of its substrate. The molecular structure and biogenesis of this ecologically fundamental organelle have remained enigmatic. The Combining Algal and Plant Photosynthesis (CAPP) collaboration aims to understand the molecular basis for pyrenoid biogenesis and leverage it to engineer a pyrenoid into land plants to enhance crop CO2 uptake and yields. In this talk, I will summarize our collaboration's progress over the past 12 years, including the discovery that the pyrenoid is a phase-separated, liquid-like organelle; the use of genetics and systematic protein localization to identify novel components of the pyrenoid; the discovery of a key linker protein that holds Rubisco together to form the pyrenoid matrix; the discovery that many pyrenoid-localized proteins share a common sequence motif that mediates targeting to the pyrenoid; and the discovery of pyrenoid membrane tubule biogenesis factors. I will also share progress on leveraging this knowledge to engineer a synthetic pyrenoid into plants.

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Abstract 1466

In Vitro Assembly of a Microcompartment from a Single Carboxysome Shell Protein

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Anthony Vecchiarelli

Bacterial microcompartments (BMCs) are a diverse and widespread class of organelles that consist of a selectively permeable protein shell that confines and increases the efficiency of specific enzymatic reactions. Native BMC shells self-assemble using multiple homologs of three protein oligomers: hexamers, trimers, and pentamers. Remarkably, thousands of copies of these proteins tile together to form the complex icosahedral BMC shell. Because BMCs are entirely protein-based and self-assemble, there is great interest in bioengineering BMCs into nanofactories and molecular scaffolds. However, the complexity of BMC shells and limited knowledge of native BMC assembly hinder current BMC-based bioengineering efforts. Therefore, studying the assembly and stability of simplified BMC architectures is imperative for the rationale design of synthetic BMCs. Here, we developed a method for the *in vitro* assembly of single-component microscale shells using only CcmK2, the major hexameric shell protein of the carboxysome BMC. While previous studies have demonstrated the ability of CcmK2 and other BMC shell proteins to form sheets and nanotubes *in vitro*, this is the first report of a single BMC shell protein making a compartment. Through microscopy and biochemical characterization, we found that CcmK2 shell size can be tightly regulated via pH during assembly. Once assembled, CcmK2 shells remain stable over broad ranges of pH and salt concentration. Lastly, we investigated CcmK2 shell encapsulation of specific and non-specific protein substrates, as well as shell permeability to these substrates. We envision these minimized microscale shells to be an important tool for synthetic biology. Determining the molecular drivers behind the assembly of these shells will inform the rational design of minimal nanofactories, molecular scaffolds, and other protein-based tools. Overall, these results advance our knowledge of the stability and modularity of BMC architectures and provide insights into how shell proteins may interact during native BMC assembly. Furthermore, because of their minimal make-up, these shells will serve as a useful tool for reductionistic investigations of protein interactions on both the interior and exterior sides of BMC shells.

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Abstract 1468

Characterization of a peroxidase-loaded encapsulin from enterobacterial pathogens

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Tobias Giessen

Intracellular compartmentalization is a fundamental feature of cells. Encapsulins (Encs) are prokaryotic protein-based nanocompartments. Encs sequester cargo proteins that encode either C-terminal targeting peptides or N-terminal encapsulation-mediating domains. A common cargo protein type associated with encapsulins are the heme-bound Dye-decolorizing Peroxidases (DyPs). Major enterobacterial pathogens, including Salmonella, Shigella, and Escherichia species, that can cause extraintestinal and gastrointestinal diseases, encode a conserved DyP-Enc operon situated inside a mobile genetic element. The molecular function of this encapsulin nanocompartment and its influence on the fitness and virulence of enteric pathogens are currently unknown. Here, we set out to (i) resolve the architecture of the cargo-loaded encapsulin, and (ii) establish the involvement of the DyP-Enc operon in enterobacterial stress response, fitness, and virulence. Using cryo-EM, the encapsulin shell and unencapsulated DyP structures were resolved at 2.5 Å and 4.3 Å resolution, respectively. The encapsulin shell assembles into 60 subunits with a diameter of 20 nm, displaying T1 symmetry. The unencapsulated DyP forms hexamers with the capacity to degrade organic and inorganic peroxides. A two-plasmid expression system was employed to determine the organization of cargo-loaded encapsulins. Through this approach, we optimize the heme-loading of DyP and DyP encapsulation. Furthermore, peroxide disc diffusion assays exhibited similar levels of increased resistance for heterologous strains expressing either the DyP-Enc operon or Enc in neutral pH environments. Although the physiological function of this peroxidase-loaded encapsulin remains to be elucidated, our results suggest it may serve as a detoxification mechanism for enterobacterial pathogens during oxidative stress.

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Abstract 1472

An inside job: Identification of a mitochondrial intermembrane space protein that works from the inside to help divide the organelle

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Olivia Connor, Srujan Matta

Mitochondria are highly dynamic organelles that maintain their shape through the mechanistically conserved processes of organelle fission and fusion. These mitochondrial dynamics are required for numerous functions, including controlling the spatial positioning of the organelle, maintenance of the mitochondrial genome, organelle quality control, and regulation of cell death. Mitochondrial fission is performed by the dynamin-related protein Drp1 (Dnm1 in yeast), a large GTPase that constricts and divides the mitochondria in a GTP hydrolysis-dependent manner. Despite over 20 years of research into the mechanisms of mitochondria fission, it has remained controversial whether factors inside mitochondria help coordinate the process and if Dnm1/Drp1 activity alone is sufficient to complete fission of both mitochondrial membranes. Here, we have identified a new factor required for mitochondrial division in yeast, which we name Mitochondrial Division Intermembrane Space 1 (Mdi1). Mdi1 is a poorly characterized, soluble protein that targets to the compartment between the mitochondrial inner and outer membranes. We find that deletion of Mdi1 leads to hyperconnected mitochondria networks, similar to loss of Dnm1. Using confocal microscopy, we find that Mdi1 localizes to discrete focal assemblies that are spatially linked to sites of mitochondrial division. However, unlike all other known division machinery, loss of Mdi1 does not inhibit Dnm1 recruitment to mitochondria. Even under conditions that promote fragmentation of mitochondria, we find Dnm1-mediated mitochondrial constriction is not sufficient to complete division of the organelle in the absence of Mdi1. Mdi1 contains a putative amphipathic helix, mutations in which lead to hyperfused mitochondria. We propose a model in which Mdi1 inserts in membranes to facilitate membrane deformation and help Dnm1 complete fission of the organelle. Thus, our work reveals that Dnm1-mediated constriction alone is insufficient to divide mitochondria without the coordinated activity of a protein that resides inside mitochondria.

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Abstract 1479

Diversity, Structure, Function and Engineering of Primitive Protein-Based Organelles: Bacterial Microcompartments

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Bacterial microcompartments (BMCs), are widespread among Bacteria; they are multienzyme-containing proteinaceous organelles bounded by a selectively permeable protein shell. One example, the carboxysome is a self-assembling metabolic module for CO2 fixation found in all cyanobacteria. These large (~100–500 nm) polyhedral bodies sequester Carbonic Anhydrase and RuBisCO within a protein shell, thereby concentrating substrates and protecting RuBisCO from oxygen generated by the light reactions. Because carboxysomes and other BMCs function to organize reactions that require special conditions for optimization, including the sequestration of substrates, cofactors, or toxic intermediates and the protection of oxygen sensitive enzymes, they have received considerable attention as templates for synthetic nanoreactors in bioengineering and as metabolic modules for programming synthetic microbial consortia. In recent survey of sequence databases we identified more 7000 BMC loci, across 45 bacterial phyla, that cluster into 68 BMC types or subtypes, including 29 new functional BMC types or subtypes. We have developed a new tool, BMC Caller, to enable researchers to identify BMCs in bacterial genomes. This will facilitate understanding of their natural functions and diversity as well as reveal new paradigms for the range of compartmentalized catalysis and bioinspiration for BMC engineering. Efforts in repurposing BMCs as metabolic modules for programming microbial metabolism and the development of nanoreactors and therapeutic devices will also be described.

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Abstract 1539

Imaging inter-organelle communication during differentiation

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Maria Clara Zanellati

Organelles undergo dramatic changes in shape, position, dynamics, and interactions with other organelles (together termed morphodynamics) to fine tune the metabolic state of a cell. Until recently, scientists lacked the tools to study organelle organization in living cells at a systems level. We have developed a method for multispectral imaging of up to eight organelles simultaneously in live cells. We used this method to study organelle dynamics along the differentiation of induced pluripotent stem cells (iPSCs) into cortical neurons (iNeurons). We labelled iPSCs and iNeurons with organelle markers and collected multispectral images at five timepoints throughout neuronal differentiation: iPSCs, and iNeurons at day 7, day 14, day 21, and day 30. Raw images were then subjected to linear unmixing and run through a Cell Profiler image analysis pipeline for segmentation and analysis of approximately 400 morpho-metrics including organelle area, size, shape, and number, as well as the number and area of organelle contacts. We found that the cell body contracts during differentiation from an iPSC to an iNeuron. Interestingly, some organelles scale with the cell body, while others do not. We also observed changes in organelle morphology. For example, tubulation of mitochondria increased as iPSCs differentiated into cortical neurons, consistent with increased oxidative phosphorylation as cells differentiate. We noted that organelle communication networks dramatically rewire throughout iNeuron differentiation. At early stages of differentiation, we observed rearrangement of mitochondria-organelle contacts, while during synaptogenesis, peroxisome-organelle contacts changed most dramatically. We found an increase in higher order contacts (3-, 4- and 5-way contacts) when neurons became mature, compared to young neurons and undifferentiated iPSCs. Our results suggest that extensive rewiring of organelle contacts is necessary to support metabolic changes throughout differentiation, and that the resulting organelle signature is required to sustain neuronal functions.

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Abstract 1570

RNA structure modulates emergent material properties of model neuronal granules

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Shana Elbaum

The translocation and processing of mRNA play a key role in neuronal development. RNA binding proteins are found abundantly in membranelles protein assemblies known as neuronal granules, which aid in mRNA trafficking within the neuron. These assemblies are enriched with Fragile X mental retardation protein (FMRP), the absence of which results in the development of Fragile X Syndrome, the most common genetic cause of autism and intellectual disabilities. FMRP is linked to translational repression and localization of mRNA. However, the binding specificity between the protein and its RNA targets is not well understood. Previous studies suggest that the low complexity region (LCR) of FMRP has a preferential binding affinity towards G-quadruplex containing mRNA. Here, we combine microrheology and fluorescence correlation spectroscopy (FCS) to examine the effect of RNA identity on the material properties of phase separated FMRP-LCR condensates. We find that distinct RNA identities can modulate the physical properties of FMRP-LCR liquid droplets as a function of secondary structure and binding affinity. The role of FMRP methylation, a common post-translational modification of the protein *in-vivo*, is further interrogated. Our findings lend insight into the mechanisms by which targeted RNA molecules modulate the material properties of neuronal granules.

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ASBMB

Abstract 1615

RNA Polymerase II Recruitment of Transcriptional Regulators Promote Phase Separation during Eukaryotic Transcription

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Joshua Mayfield, Jack Dixon, Y. Jessie Zhang

The recent discovery of cellular liquid-liquid phase separation emerges as a new membraneless organelle, identified in various cellular processes. Various condensates can co-exist in the same biological process. One critical question yet to be fully addressed is how the different condensates prevent phase fusion and how the same biomolecule enters, exits, and migrates to various droplets. Here, we investigate the intrinsically disordered region of RNA polymerase II and identify what leads it to change phase states. The reversible phosphorylation of the CTD by enzymatic activities of kinases/phosphatases dissolve condensates formed by unphosphorylated CTD due to the negative-charge repulsion, where the location of the charges or the identity of the negative charge group has little impact. With phosphoryl-CTD homogenous in solution, its association with prolyl isomerase 1 (Pin1) causes the condensate to form. Such effect is not due to Pin1's enzymatic ability to change prolyl configuration, but its association with the phosphorylated CTD. This finding extends to other phosphoryl-CTD binding proteins, in which the association neutralizes the charge and allows the condensates to reform. Intriguingly, the condensates formed by phosphoryl-CTD and associated proteins have different physical properties from those formed by unphosphorylated CTD, allowing them remain separated without fusion even when located right next to each other because they are governed by different physical interactions. In cells, when the CTD recruits binding proteins to RNA polymerase II, the transcription regulators and factors are sorted automatically in different condensation states in cells based on the governing biophysical forces and their interaction with the core proteins of the condensates.

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Abstract 1648

Overexpression of the citrate transporters SLC13A5 and SLC25A1 leads to different phenotypes in the mouse

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Katherine Overmyer, Alexis Lawton, Ildiko Kasza, Patricia Gallego-Muoz, Joshua Coon, John Denu, Caroline Alexander, Luigi Puglielli

Background: Import of acetyl-CoA into the endoplasmic reticulum (ER) lumen by AT-1/SLC33A1 regulates the proteostatic functions of the organelle and the engagement of the secretory pathway. Homozygous and heterozygous loss-of-function mutations affecting AT-1 are associated with developmental delay and hereditary forms of sensory and autonomic neuropathy, while gene duplication of AT-1 is associated with autism spectrum disorder (ASD), intellectual disability, and dysmorphism. Mice with reduced or increased AT-1 activity mimic associated human diseases and manifest defects in ER proteostasis and dynamics of the secretory pathway. By analyzing these animals, we discovered that AT-1 acts in conjunction with SLC25A1 and SLC13A5 to maintain intracellular acetyl-CoA flux and metabolic connectivity. SLC25A1 is a mitochondria membrane transporter; it transfers citrate from the mitochondria lumen to the cytosol. SLC13A5 is a plasma membrane transporter; it transfers citrate from the extracellular milieu to the cytosol. In the cytosol, citrate is converted to acetyl-CoA by ACLY, and serves as donor of the acetyl group for Nε-lysine acetylation within the cytosol, the nucleus, and -following AT-1 mediated translocation- the ER lumen. As with AT-1, gene duplication events of SLC25A1 and SLC13A5 are also associated with ASD and intellectual disability; furthermore, haploinsufficiency of SLC13A5 homologues in *D. melanogaster* and *C. elegans* increases life span.

Results: Here we report the generation of mice with systemic (sTg) overexpression of either SLC25A1 or SLC13A5. SLC13A5 sTg mice were born with Mendelian ratio but died immediately after birth. In light of the early mortality of SCL13A5 sTg, we also generated mice where the overexpression of SLC13A5 was induced either at birth or at weaning. In both cases, the animals developed a progeria-like phenotype that resembled AT-1 sTg. In contrast, SLC25A1 sTg mice were born with Mendelian ratio and displayed normal lifespan. Both animals displayed increased cytosolic levels of citrate and acetyl-CoA. However, analysis of the metabolic profile of SLC13A5 and SLC25A1 sTg mice revealed widespread differences. Furthermore, SLC13A5 sTg mice presented increased levels of endogenous AT-1 while SLC25A1 sTg mice did not, suggesting that the functional adaptation of the ER and consequent alteration in ER proteostasis might underlie the phenotypic differences. Consistently, pharmacologic rescue of the proteostatic functions of the ER was able to improve the progeria-like phenotype of SLC13A5 sTg mice.

Conclusions: In conclusion, our findings suggest that altered intracellular citrate/acetyl-CoA flux as caused by increased SLC13A5 and/or AT-1 activity maintains real-time crosstalk between intracellular organelles and compartments, and influences ER proteostasis. Our findings also point to different biological responses to the overexpression of SLC13A5 and SLC25A1.

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Abstract 1707

Stress Granule Quality Control Mechanisms by the Endoplasmic Reticulum

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Jason Lee

Stress granules are membraneless organelles that form in response to cellular stresses to inhibit bulk mRNA translation. When stress is no longer present, stress granules disassemble over the course of 90 minutes to resume mRNA translation, however the quality control mechanisms that underlie the disassembly process remain unclear. Understanding how stress granules are efficiently disassembled is important because chronically persistent stress granules have been linked to protein aggregation and neurodegenerative disease. It was recently discovered that the endoplasmic reticulum (ER) tubules form contact sites with stress granules during stress granule fission. Although stress granule fission and fusion events are frequently observed, there remains a key knowledge gap in our understanding of how stress granule fission contributes to disassembly. In this study, I combined approaches in molecular biology and cell biology to reveal that ER-driven stress granule fission plays an important role in the stress granule disassembly process. Furthermore, my work uncovers a novel relationship between ER morphology and stress granule disassembly, such that altering the abundance of ER sheets results in the persistence of stress granules hours after the removal of stress. Since smaller condensates have been shown to exchange components at a higher rate with the dilute phase compared to larger condensates, this study suggests that ER-associated stress granule fission is a mechanism to generate smaller, more dynamic stress granules, which are easier to dismantle. Collectively, I propose that stress granule fission works in concert with dissolution to efficiently disassemble stress granules.

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Abstract 1755

A Role for SLC25A46 in Mediating Mitochondrial Dynamics

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Jordan Tibbs, Janos Steffen, David Austin, Nicole Zagon, Ryan Howe, Robert Damoiseaux, Alex van der Bliek, Carla Koehler

Mediation of mitochondrial dynamics is essential to the health of an organism and its cells. If this process is disrupted, several diseases can result, including Pontocerebellar Hypoplasia (PCH), Charcot-Marie-Tooth Disease Type 2 (CMT2), and Optic Atrophy. Maintenance of a healthy mitochondrial network relies on a delicate balance between mitochondrial fission and fusion. The two processes must be coordinated across the inner and outer mitochondrial membranes, but how this occurs is not very well understood. Recently, we have identified a protein, SLC25A46, that may be responsible for coordinating the two processes. The protein is a member of the Solute Carrier Family but is localized to the outer mitochondrial membrane unlike the other solute carrier proteins, which are localized to the inner mitochondrial membrane. The protein has been shown to interact with several proteins including fusion regulators Mitofusin 1+2 (MFN1+2) on the outer membrane and Dynamin-like 120 kDa Protein (OPA1) on the inner membrane along with Mitochondrial Fission Factor (MFF) and several members of the Mitochondrial Cristae Organizing System (MiCOS) complex. When expression of SLC25A46 is downregulated, expression of MFN1+2 is increased and a hyperfused mitochondrial network is observed. The same phenotype results when the gene encoding the protein undergoes a point mutation at Position 341 from leucine to proline (L341P). The misfolded protein is properly imported into the mitochondria, but is promptly identified, extracted, and degraded by the ubiquitin-proteasome system (UPS), which is made up of the redundantly-functioning E3 ubiquitin ligases MULAN and March5, the ATPase p97, and the proteasome. This process occurs independently of mitophagy, making SLC25A46 L341P a model substrate to study the process of Outer Mitochondrial Membrane Associated Degradation (OMMAD.) In order to study the underlying mechanisms of OMMAD, this project utilizes Genome-wide CRISPR screening methods to knock out expression of individual genes in single cells and study the observed phenotypes to determine whether such genes function to coordinate or regulate this process. SLC25A46 has recently been hypothesized to have a competing role with Mitochondrial Carrier Homolog 2 (MTCH2 aka SLC25A50), as both proteins belong to the solute carrier family and are found on the mitochondrial outer membrane yet have no known transporter function. MTCH2 has a known role in regulating lipid metabolism, as downregulation results in lower levels of lipid droplet formation and a fragmented mitochondrial network. When SLC25A46 undergoes a L341P mutation, MTCH2 expression levels are increased. However, changes in SLC25A46 expression do not appear to have a direct effect on levels of lipid droplet formation. It is possible that SLC25A46 may act as a sensor for metabolic shifts between glycolysis-based and oxidative phosphorylation-based metabolism, so this project characterizes changes in mitochondrial morphology when cells are treated with excess succinate to simulate a block in the TCA Cycle, necessitating the shift from oxidative phosphorylation to glycolysis. This project seeks to determine the role of SLC25A46 in mediating mitochondrial processes and how deficiencies in its function can result in disease states.

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Abstract 1785

The role of phase separation in the control of TAF15-mediated transcriptional activation

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Ilmin Kwon

As a member of the transcription factor IID complex, TAF15 is involved in the control of transcription at distinct promoters. Here, we show that the low-complexity (LC) domain of TAF15 undergoes phase separation into liquid-like droplets *in vitro*, and this process is critical for TAF15 to function as a transcriptional regulator in cells. Phosphomimetic substitutions of the serine residues in the LC domain prevented phase separation and transcriptional activity of TAF15, suggesting that serine phosphorylation may be a key regulator of TAF15 phase properties thus modulating its transcriptional activity. In addition, mutational analysis interrogating serine or tyrosine residues indicates that the phase separation capacity of the TAF15 LC domain is required for binding to different kinds of RNA binding proteins that enhance TAF15-induced transcriptional activation. These results provide a framework for understanding the regulatory mechanism of TAF15 phase separation and how this process contributes to TAF15-mediated transcription.

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Abstract 1865

Overview of the ryanodine receptor calcium Ion channel and the diverse interactions it has with calcium, S-adenosyl-L-methionine, and the family of adenine nucleotides

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Vikram Murugesan, Alana Bennett, Laura Chen, Anne Roundhill, Alena Wolfe-Tham

The ryanodine receptor (RyR) is a massive ligand-gated ion channel of about 2.24 million daltons, with three major isoforms (RyR1, RyR2, and RyR3). Each ryanodine receptor is a homotetramer that sits in the endoplasmic or sarcoplasmic reticulum in various cells, including smooth, skeletal, and cardiac muscle cells, and releases calcium (Ca2+) to act as a secondary messenger for various cellular signaling pathways. Most notable of these pathways is excitation-contraction coupling, where transverse-microtubule voltage changes signal the opening of the RyR and release of calcium into the cell cytosol, resulting in muscle contraction. Additionally, RyRs have a large number of modulatory ligands, which have a variety of effects on the RyR's conformational states and conductance. In this review, we explore the basic structures and functions of Ryanodine Receptor 1 (RyR1) and Ryanodine Receptor 2 (RyR2) and current knowledge about their various regulatory ligands, including Ca2+, ATP and various adenine nucleotides including S-adenosyl-L-methionine (SAM). Additionally, we will describe the observed interactions each of these ligands has with the RyR, with special emphasis on the voltage-dependent and conductance-influencing effects of SAM, and explain the proposed mechanisms of these interactions. Finally, we will use the 3-dimensional chemical viewing program JMOL and 3D printing to represent the overall structure, conformational changes, and proposed ligand interactions of the RyR as discussed in our secondary research.

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Abstract 1888

The protein optic atrophy 1 (OPA1) functions as a novel assembly factor for the respiratory complex V, and its deletion is tolerated in the liver

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Tae Jin Lee, Chad Galloway, Wenbo Zhi, Karen Bentley, Ashok Sharma, Hiromi Sesaki, Yisang Yoon

The liver is involved in the multitude of processes including the metabolic homeostasis and detoxification. Mitochondria are critical for these functions, and thus, mitochondrial dysfunction is one of the main causes for liver diseases. Mitochondrial fission and fusion determine mitochondrial morphology, and are important for maintaining mitochondrial function. The optic atrophy 1 (OPA1) protein is one of the mitochondrial dynamics proteins that mediates fusion of the inner membrane. OPA1 was also shown to be important for maintaining the cristae structure. Hence, the OPA1 gene disruption has been shown to impair mitochondrial electron transport and ATP production. However, the role of OPA1 in liver function is poorly understood. In the current study, we acutely deleted OPA1 in the mouse liver and examined its effect. All animal experiments were performed according to procedures approved by the IACUC at Augusta University. We introduced adeno-associated virus (AAV) that carries hepatocyte-specific Cre (AAV8-TBG-Cre) to OPA1-floxed mice at the age of 8 weeks. Cre was expressed for 8-12 weeks to allow sufficient time for OPA1 deletion to reveal functional consequences. AAV8-TBG-GFP was used for generating control mice. Unexpectedly, OPA1 liver knockout (LKO) mice were healthy, showing no ill phenotypes and unaffected mitochondrial respiration despite disrupted cristae structure. We found that OPA1 LKO induces alterations in liver and mitochondrial proteomes. Ingenuity Pathway Analysis of the proteomics data indicated that OPA1 KO activated sirtuin signaling, TCA cycle, growth factor/hormone signaling, and PGC1α, suggesting the effort to support mitochondrial function, mitochondrial biogenesis, and cell survival. OPA1 KO decreased EIF2 signaling, LXR/RXR signaling, lipid degradation, and xenobiotic metabolisms, suggesting general attenuation of liver activity. Importantly, by analyzing respiratory complexes, we identified a new role of OPA1 as an assembly factor for respiratory complex V. In OPA1-KO mitochondria, we observed marked accumulations of sub-complexes of complex V, demonstrating that OPA1 plays a role in proper assembly of complex V. Further analyses indicate that OPA1 is necessary for late maturation of the membrane-intrinsic Fo complex. In conclusion, we identified a new role of OPA1 in facilitating the assembly of the respiratory complex V. Inhibiting this function in the liver accumulates unassembled complex V sub-complexes, causing a mitochondrial stress to alter mitochondrial and liver proteomes to establish a new homeostatic state for sustained maintenance of mitochondrial and liver functions.

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Abstract 1963

Reflectin protein shows length-dependent condensate and particulate gel formation in HeLa cells

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Susan Walsh, Robert Levenson

Loliginid squids possess a dynamically tunable protein called reflectin, which drives skin color changes for purposes such as camouflage and communication. Reflectins are block copolymer proteins consisting of highly conserved domains interspersed with cationic linkers. Previous experimental results and reflectin's amino acid composition show that the reflectins are intrinsically disordered and have the potential to form biomolecular condensates via liquid-liquid phase separation. To evaluate determinants of reflectin assembly within eukaryotic cells, we expressed fluorescent protein fusions of full-length and truncated forms of Doryteuthis opalescens reflectin A1, along with other reflectins, in HeLa cells and imaged them with fluorescence microscopy. Reflectins demonstrated a length-dependent propensity to assemble into particulate gels or aggregates versus forming discrete puncta, wherein longer constructs composed of greater numbers of domain-linker repeats had an increased tendency to form gel-like assemblies. Length-dependent differences in cellular localization were also observed across different reflectin truncations. Our data are valuable for deepening our understanding of how the sequence of reflectin encodes its assembly properties and may provide insight into the distinct roles of different reflectin proteins in the formation of tunable iridescent nanostructures in squid.

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Abstract 2104

O-GlcNAc regulates mitochondrial integrated stress response by regulating Activating Transcription Factor 4

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The accumulation of dysfunctional mitochondria is closely associated with age-related neurodegenerative diseases (ND) including Parkinson's disease (PD) and Alzheimer's disease (AD). Impairment of mitochondrial quality control mechanism leads to the accumulation of damaged mitochondria and increasing neuronal stress. Our data established an essential role of O-GlcNAcylation, a single sugar post-translational modification, in controlling mitochondrial stress-induced transcription factor Activating Transcription Factor 4 (ATF4). Mitochondrial dysfunction triggers the integrated stress response, in which the O-GlcNAcylation and phosphorylation of eukaryotic translation initiation factor 2α (eIF2α) results in the translation of the transcription factor 4 (ATF4). Our data show that sustained OGA inhibition by Thiamet-G (TMG) treatment in SH-SY5Y neuroblastoma cell-lines elevates ATF4 protein level in total cells and in mitochondria fractions. Mitochondrial chaperone glucose-regulated protein 75 (GRP75), an ATF4 target gene, is also significantly elevated in mitochondria fraction. Interestingly, manipulating both O-GlcNAcase (OGA) and O-GlcNAc transferase (OGT) enzymes, which dynamically remove and add the sugar respectively, by knock-down (KD) increase ATF4 protein and mRNA level. Activating Transcription Factor 5 (ATF5), an ATF4 target gene, is significantly elevated in both protein and mRNA level in OGT KD SY5Y. Additionally, intra-peritoneal TMG injection for a period of six months increased ATF4 and GRP75 level in mitochondria fractions isolated from mice brains. However, one-month TMG injection have a slight induction of ATF4 and GRP75. *In vitro* model of AD shows a significant induction of ATF4 and GRP75 in TMG treated organoids. These results indicate that altering O-GlcNAc homeostasis by either inhibiting OGA, OGT KD, and OGA KD initiate mitochondrial stress by regulating ATF4.

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ASBMB

Abstract 2116

Rogdi is the functional homolog of yeast Rav2 and a potential Rabconnectin-3 subunit

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V-ATPases are highly conserved proton pumps which undergo a process called reversible disassembly to control both assembly state and catalytic activity. This process is best understood in yeast (*S. cerevisiae*) but also occurs in higher eukaryotes, including humans. The RAVE complex is responsible for promoting V-ATPase reassembly in yeast, and Rabconnectin-3 complexes play a similar role in mammalian cells. While there is substantial structural and sequence homology between the RAVE and Rabconnectin-3 complexes, their similarities and differences are not completely understood. Rav2, a functionally essential subunit of the yeast RAVE complex, does not have an identified homolog in Rabconnectin-3. Through protein modelling we identified Rogdi as a potential human homolog of yeast Rav2. The models of Rav2 generated by Phyre2 and AlphaFold2 are very similar to the experimentally derived Rogdi structure. We previously showed experimentally that Rav2 binds to the N-terminal β-propeller region of Rav1. AlphaFold-Multimer models suggest that Rav2 and Rogdi can interact with the N-terminal β-propeller of Rav1, suggesting functional overlap. Consistent with the model, Rogdi shows two-hybrid interactions with the N-terminal β-propeller of Rav1, as well as the structurally homologous N-terminal β-propeller regions of human Rabconnectin-3 subunits. Rav2 is indispensable for RAVE function in yeast, and overexpression of Rogdi in a rav2Δ mutant partially rescues the growth phenotypes characteristic of strains lacking RAVE function. This rescue is lost in the absence of Rav1, suggesting that Rogdi does not bypass RAVE function. Isolated vacuoles from a strain overexpressing Rogdi in place of Rav2 exhibit increased activity relative to a rav2Δ mutant. Mutations in Rogdi are associated with Kohlschutter Tonz syndrome (KTS), which is characterized by seizures, spasticity, amelogenesis imperfecta, and progressive dementia. Significantly, the neurological symptoms of KTS are similar to other syndromes associated with loss of Rabconnectin-3 function. Taken together, the in-silico modelling, experimental data in yeast, and phenotypic similarity suggest that Rogdi is the human homolog of yeast Rav2 and facilitates V-ATPase reassembly. Improving understanding of the mechanisms behind V-ATPase reversible disassembly will provide new avenue for the development of drugs and treatments for conditions where V-ATPases play a central role.

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Abstract 2215

Human metapneumovirus recruits purine biosynthesis enzymes to phase-separated compartments to regulate viral replication

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Human metapneumovirus (HMPV) is a respiratory virus that causes severe pathology in children, elderly, and immunocompromised individuals. It is a member of the Mononegavirales order, which contains non-segmented negative-stranded RNA viruses (NNSVs) such as measles, Nipah, and Ebola viruses. Replication and transcription of NNSVs occurs in phase separated compartments termed inclusion bodies (IBs). Formation of these phase-separated regions is driven by the phosphoprotein (P), which in combination with the nucleoprotein (N) and the viral RNA dependent RNA polymerase (L), carries out replication and transcription using the same negative-sense RNA genome. L, N, and P are sufficient to induce viral RNA replication and transcription *in-vitro* and in-situ. However, it is not known how NNSVs switch between replication or transcription. Previous work shows that the pneumovirus polymerase L utilizes local GTP and ATP levels to switch between replication and transcription *in-vitro*. Therefore, HMPV's IBs may allow for local changes in nucleotide concentrations through the use of purine biosynthesis enzymes to regulate replication and transcription over the course of infection. Our data suggests that GTP concentration increases in-situ over the early stages of infection. Metabolomics analysis of infected cells also shows an increase in numerous metabolic precursor molecules, including ribose 5-phosphate, a substrate for purine synthesis. Additionally, mRNA transcripts of rate-limiting purine biosynthesis enzymes Inosine Monophosphate Dehydrogenase (IMPDH) and Adenylsuccinate Synthetase (ADSS) increase during infection, further showing that nucleotide synthesis may be upregulated during infection. IMPDH and ADSS were seen in proximity or colocalized in HMPV IBs using immunofluorescence confocal microscopy. Further examination of the de novo GTP synthesis pathway showed GMP Synthase (GMPS) localize in IBs as well, alluding to a proteomic switch of local nucleotide concentrations. IMPDH, GMPS, and ADSS knockdown using shRNAs slowed viral replication and transcription in cell culture. Additionally, replication and transcription were also inhibited in a dose-dependent manner by ADSS and IMPDH inhibitors

when a minigenome system was used. Use of inhibitors for IMPDH also showed increased viral replication and decreased viral transcription in a dose-dependent manner. Together, these findings suggest that recruitment of purine biosynthesis enzymes to IBs may be a key component for modulating viral replication and transcription.

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Abstract 2350

Loss of Sting in parkin mutant flies suppresses muscle defects and underlying mitochondria damage through induction of pro-survival genes

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The molecular causes which induce neurodegeneration during the pathogenesis of Parkinson's Disease remains to be fully understood, yet early intervention may lead to novel treatment and prevention strategies. In the model organism *Drosophila melanogaster*, loss of the early-onset PD gene parkin (the ortholog of human PRKN) results in visible robust phenotypes, including impaired climbing ability, damage to the indirect flight muscles, and mitochondrial fragmentation with swelling. The presence of mitochondria damage with impaired mitochondrial quality control mechanisms has been proposed to activate innate immune pathways through release of damage associated molecular patterns (DAMPs). In mammalian models, PRKN-mediated mitophagy is hypothesized to suppress mitochondrial DAMP release and subsequent activation of the conserved cGAS-Sting innate immunity pathway, but the role of these conserved innate immune pathways in the fly model remains unresolved. Using genetic models, quantitative phenotyping, confocal microcopy, and RNA-sequencing approaches, the effects of Sting deletion in parkin and pink1 mutant flies was tested. Our findings demonstrate that loss of Drosophila Sting rescues the thorax muscle defects and the climbing ability of parkin-null mutants. Loss of sting also partially suppresses the disrupted mitochondrial morphology in parkin flight muscles, suggesting unexpected feedback of Sting signaling on mitochondria integrity or indirect activation of compensatory pathways. In the parkin and sting double mutant animals the ubiquitin kinase Pink1, an upstream mediator of Parkin, is strongly activated, however loss of sting had no significant effect on phenotypes in pink1 mutants. TUNEL detection assays indicate that the apoptosis observed in parkin mutant muscle is suppressed in flies lacking both parkin and sting. Analysis of enriched transcripts in these double mutant flies supports a revised hypothesis that an increase in oxidative stress-responsive pathways, such as glutathione-S-transferase enzymes and cytochrome p450 family members, suppresses oxidative damage from impaired mitochondria. These findings support a novel, non-canonical role for Drosophila Sting in the cellular and organismal response to mitochondria stress.

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ASBMB

Abstract 2384

Lessons Learned from Bacterial Microcompartments: Self-Assembly, Architecture, Biosynthesis and Bioengineering

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Intracellular compartmentalization and self-assembly of proteins into large supercomplexes underpin most biological activities in living organisms. Bacterial microcompartments (BMCs) are a paradigm of proteinaceous organelles widespread in prokaryotes. These nanoscale organelles (typically 100–400 nm in size) sequester key enzymes and pathways in the cytoplasm to enhance metabolic performance. Based on their functions, BMCs can be divided into carboxysomes, which serve as the key CO2-fixing organelles in cyanobacteria and many proteobacteria, and metabolosomes in pathogenic bacteria for the breakdown of metabolites such as propanediol and ethanolamine. The natural self-assembly and architectural features of BMCs have attracted increasing interest in repurposing BMC structures for biotechnological and biomedical applications in metabolic improvement, molecule delivery and therapeutics using synthetic biology. In this seminar, I will present our recent studies on (1) the self-assembly and biosynthesis principles of BMCs, and (2) the bioengineering of carboxysome structures in heterologous organisms (such as *E. coli* and plants) for improved metabolism and bioenergy production, harnessing the knowledge that we learned in nature.

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Abstract 2448

Evaluation of peroxisome size regulation through optogenetic enlargement for future use as synthetic organelle in *Saccharomyces cerevisiae*

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Mark Chan, Jordan Baker, John Dueber

Emerging research on artificial cells and organelles shows great potential for refined production of biomolecules including pharmaceuticals. Synthetic organelles are at the forefront for deeper understanding and control of cellular reactions. In this poster, we focus on the peroxisome that has previously shown to be capable of compartmentalizing heterologous proteins, a necessary ability for usage as a synthetic organelle. Previous studies have shown that the amount of protein able to be imported into the peroxisome depends on the organelle's size, so we aim to engineer cells with larger peroxisomes to maximize capacity. Furthermore, initial studies have shown that the size of organelles typically scales closely with cell size. Therefore, we aim to produce larger peroxisomes in budding yeast by applying optogenetic perturbations to grow cells to artificially large sizes. Preliminary results showed that in these enlarged cells, peroxisomes do maintain a scaling relationship with cell size, and this occurs via proliferation rather than expansion in volume. Future experiments will use mutants targeting peroxisome biogenesis, fission, and fusion pathways to gain more mechanistic insight into how peroxisome size is regulated.

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Abstract 2457

A Mitochondria-ER-PM contact site regulates the distribution of phosphatidylinositol-4-phosphate

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Membrane contact sites (MCSs) are sites of close membrane apposition that tether organelles to organize their intracellular distribution and facilitate the exchange of biological materials. In yeast, the mitochondria-ER cortex anchor (MECA) is a tripartite MCS between the plasma membrane (PM), the ER, and mitochondria. The core component of MECA, Num1, is required for the cortical distribution of the mitochondrial network and also functions in nuclear inheritance by serving as an anchor for dynein. Previous work has shown that Num1 interacts with the PM and mitochondria via two distinct lipid binding domains; however, the molecular mechanism by which Num1 interacts with the ER is unclear. Here we demonstrate that the two phenylalanines in an acidic tract (FFAT) motif in the C-terminus of Num1 interacts with the integral ER membrane protein Scs2. Deletion of the FFAT motif disrupts Num1-ER localization but is dispensable for mitochondrial tethering and dynein anchoring. Unexpectedly, IP-MS experiments revealed that MECA interacts with components of the PM-localized phosphoinositide kinase (PIK) patches which synthesize phosphatidylinositol-4-phosphate (PI(4)P). We found that MECA both colocalizes and has a synthetic genetic interaction with PIK patch components. Remarkably, loss of Num1-mediated mitochondrial tethering or the Num1-Scs2 interaction caused a significant change in the distribution of PI(4)P. These data support a model where MECA serves as an organizational hub that regulates the cellular distribution of organelles as well as the lipids critical for their functions.

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Abstract 2520

Using computer simulations to uncover the molecular grammar of biomolecular phase separation

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The formation of membraneless organelles (MLOs) via phase separation of proteins and nucleic acids has emerged as an essential process with which cells can maintain spatiotemporal control. Despite enormous progress in understanding the role of MLOs in biological function in the last ten years or so, the molecular details of the underlying phenomena are only beginning to emerge recently. We use computer simulations of coarse-grained (CG) and all-atom (AA) models to complement experimental studies to achieve insights into the molecular driving forces underlying biomolecular phase separation. In this talk, I'll specifically focus on the development of CG computer models and multiscale simulation techniques aimed at elucidating the sequence-determinants of biomolecular assembly. I'll also highlight results that demonstrate our approach's usefulness for identifying general principles and system-specific insights into biomolecular structure and function.

Our work on biomolecular phase separation is supported by the National Institutes of Health grants R01NS116176, R01GM136917, Welch Foundation grant A-2113-2022033, and National Science Foundation grant DMR2004796.

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Abstract 2530

The role of low complexity repeats in the evolution, structure and function of biomolecular condensate

Eliezer Calo, *Massachusetts Institute of Technology*

A guiding principle of biology is that biochemical reactions must be organized in space and time. Liquid-liquid phase separation has emerged as a physicochemical mechanism with the potential to explain how cells efficiently execute and organize biochemical reactions. Many intracellular bodies and signaling assemblies are assembled via liquid-liquid phase separation but the biochemical and molecular details behind their assembly and architecture are unknown. One such assembly is the nucleolus, the ribosome factory of eukaryotic cells. I will illustrate how my lab leveraged the relationship between protein sequences and the assemblies they form to study the underlying physical and chemical principles that generate the internal architecture of the nucleolus. I will also provide insights into how a single protein, TCOF1, is responsible for the evolutionary origin of a nucleolar assembly, called the fibrillar center, and its role in development and disease.

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Abstract 2553

Tunable properties and dynamics of biomolecular condensates

Shana Elbaum, *ASRC*

Proteins, and other types of biomolecules, can self-assemble into materials with an incredible range of physical properties exquisitely tied to their physiological function – from the elasticity of elastin fibers in the heart and lungs to the dynamics of stress granules and other membraneless organelles. Despite their functional significance, there remain substantial gaps in understanding how individual proteins and/or nucleic acids come together on the molecular scale to produce unique properties on the material mesoscale. Here, we leverage rheological and quantitative fluorescence approaches to extract fundamental principles governing protein self-assembly into "condensates" with unique viscoelastic properties, dynamics and hierarchical organization. Utilizing reductionist model polymer systems, we specifically examine how arginine and lysine residues, and their post-translational modifications, contribute to unique material properties and architectures. This work lends mechanistic insight into the growing number of arginine/lysine rich phase separating protein domains implicated in a vast set of biological processes.

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Abstract 2555

Functional reconstitution of the bacterial CO2 concentrating mechanism

David Savage, *University of California, Berkeley/HHMI*

Many autotrophs rely on biophysical CO2 concentrating mechanisms (CCMs) to assimilate carbon. It is postulated that principles - and perhaps even components – of bacterial CCMs could be used to improve CO2 assimilation in plants. Surprisingly, defining a systematic 'parts list' of the bacterial CCM remains an open question. To this end, we have carried out a genome-wide barcoded transposon screen to identify essential and CCM-related genes in the γ-proteobacterium *H. neapolitanus*. Screening revealed that the CCM comprises at least 17 and likely no more than 25 genes, most of which are encoded in 3 operons. Found within these genes is a new class of Ci pump which we term DAB, for 'DABs accumulate bicarbonate,' that is widespread amongst prokaryotes and readily expressed in a heterologous fashion. Informed by this information, we have also performed a functional reconstitution of the bacterial CCM *in vivo*. Using a novel strain of Rubisco-dependent *E. coli*, we have assessed the importance of known and poorly characterized activities of the CCM for achieving efficient CO2 assimilation and, ultimately, have engineered a strain which is capable of growth due to efficient CO2 fixation directly from ambient air.

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Abstract 2569

Monatomic Ions Influence Substrate Permeation Across Bacterial Microcompartment Shells

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Bacterial microcompartments (BMCs) are protein organelles consisting of an enzymatic core encased within a selectively permeable shell. BMCs shells are modular, tractable architectures that can be repurposed with new interior enzymes for biomanufacturing purposes. The permeability of BMC shells is function-specific, and it is regulated by biophysical properties of the shell subunits, especially its pores. Many BMC shell proteins are highly charged at their pore interface. Accordingly, the permeation of substrates and ions have been investigated but their influence on one another has yet to be delineated. We speculated that ions may interact with pore residues in a manner that affects the substrate permeation process. We tested this hypothesis by monitoring the activity rates of native 1,2-propanediol utilization (Pdu) BMCs in different NaCl concentrations. This approach served as a proxy for directly measuring permeation events in our model BMC. Here, the rate of 1,2-propanediol conversion to propanal was compared between native and broken Pdu BMCs under different NaCl concentrations to evaluate the role of the outer shell in mediating that process. Molecular dynamics simulations were then used to assess the mechanism by which monatomic ions affected permeation. Our *in vitro* activity comparisons between native and broken BMCs demonstrated that increasing NaCl negatively affects permeation rates. Molecular dynamics simulations of the dominant shell protein (the BMC-H PduA) revealed that chloride ions preferentially occupy the positive pore, hindering substrate permeation, while sodium ions remain excluded. In contrast, 1,2-propanediol was not found to significantly influence chloride or sodium permeation. We believe our observations with chloride ions are extrapolatable to other anionic species such as phosphate and bicarbonate and may influence permeation in related BMCs. Overall, these results demonstrate that shell properties influence ion permeability, and leverages molecular dynamics to improve our understanding of BMC shells towards their repurposing for biotechnological applications.

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Abstract 2587

Reconstitution of the calcium-triggered contractile apparatus of the ciliated protozoa Spirostomum

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Tyler Bartolome, Fritz Melzl, Connor O'Shea, Saad Bhamla

Protists, notably ciliated protozoa, exhibit a novel form of contraction that is rapidly triggered by calcium ions but does not require ATP hydrolysis. Ultrafast forms of calcium-triggered contraction have been described for the Vorticella stalk and the Spirostomum cell body. In heterotrich ciliates Stentor and Spirostomum, contraction is mediated by branching, spindle-shaped structures called myonemes. The goal of these studies is to reconstitute this novel contractile system *in vitro*. Through mass spectrometric analysis of Spirostomum ambiguum cytoskeletal preparations, a family of EF-hand proteins closely related to the calcium-binding protein centrin has been identified in several Spirostomum species. Bioinformatics analysis of published and derived proteome data suggests the existence of a distinct subgroup of centrin-like calcium-binding contractile proteins in a variety of ciliated protozoa. In addition, several candidates for a centrin-binding Sfi1-like scaffold protein have been identified in the Spirostomum proteome. Synthetic genes encoding Spirostomum centrin and Sfi1-like centrin-binding proteins have been engineered for expression in *E. coli*. Expression of the Sfi1-like repeat protein in bacteria yielded little protein, while co-expression of Spirostomum centrin and a single Sfi1-like repeat protein yielded both proteins in milligram quantities. These proteins have been purified by IMAC and gel filtration chromatography. Gel filtration chromatographic separations revealed a single peak containing the two proteins, with a molecular mass consistent with the formation of a 1:1 complex. Electrophoretic mobility shift experiments showed that both centrin and the centrin-Sfi1 complex undergo a conformational change upon adding calcium. Microscopic observations revealed that concentrated mixtures of these two proteins assembled to form unusual branching networks when calcium was added. Another version of the Sfi1-like protein has been engineered with three repeats, which appears to bind centrin in the expected 1:3 stoichiometry. Current efforts are aimed at engineering a self-assembling form of centrin-Sfi1 filament bundles that can be induced to contract in response to calcium ions. Our ultimate goal is to engineer Spirostomum contractile proteins to fully reconstitute *in vitro* the ultrafast form of contraction seen in living Spirostomum cells. These studies should yield insight into the structure, mechanism, and regulation of these novel contractile systems in living cells.

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Abstract 2639

Pharmacological control of peroxisome biogenesis

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Peroxisomes are highly inducible intracellular organelles, responding to fats, hypolipidemic agents and nongenotoxic carcinogens, and during processes of development and differentiation. Peroxisome proliferation involves signal recognition and computation by molecular networks that direct gene expression, metabolism, membrane biogenesis, proliferation, protein import, and organelle inheritance. Yet how peroxisome proliferation is controlled in humans is unknown. We characterized the peroxisomal response of primary human hepatocytes to a panel of 38 broadly acting kinase inhibitors. Quantitative measurements of peroxisomes and, specifically, two peroxisomal proteins, Pex3 and Pmp70, were made via high-throughput microscopy. Distinct kinase inhibitors impacted peroxisome biogenesis, by either increasing or decreasing peroxisome numbers and/or altering the localization of Pex3 between the peroxisome and the endoplasmic reticulum. Machine learning algorithms are currently being used to 'deconvolve' the dataset to make predictions of specific kinases that control peroxisome biogenesis. These predictions are being experimentally validated. Thus, we demonstrate pharmacological control of peroxisomes in primary human cells, an important first step for understanding how peroxisome biogenesis is controlled in humans.

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Abstract 2699

Unique interactions and functions of the mitochondrial small Tims in *Trypanosoma brucei*

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Linda Quinones, Fidel Soto-González, Chauncey Darden, Muhammad Khan, Anuj Tripathi

Trypanosoma brucei, that causes African trypanosomiasis, possesses a unique translocase of the mitochondrial inner membrane, the TbTIM17 complex, which imports hundreds of nuclear-encoded mitochondrial proteins. TbTim17, the major component of this complex associates with 6 small TbTims, (TbTim9, TbTim10, TbTim11, TbTim12, TbTim13, and TbTim8/13). Like small Tim homologues in other eukaryotes, each of these small TbTims possesses a characteristic helix-loop-helix structure with a pair of disulfide bonds, except for TbTim12. Small TbTims are essential for *T. brucei* cell growth and the stability of the TbTIM17 complex. However, interaction pattern of the small TbTims and their functions are not completely explored in *T. brucei*. Here we demonstrated by Yeast-2-hybrid (Y2H) analysis that all six sTbTims directly interact with each other, however, stronger interactions were found among TbTim8/13, TbTim9, and TbTim10. Most of the small TbTims directly interact with the C-terminal hydrophilic regions of TbTim17 strongly. Each small TbTims co-immunoprecipitated TbTim17 and other small TbTims from *T. brucei* mitochondrial extract. However, TbTim10 showed stronger affinity for TbTim9 and TbTim8/13, and least for TbTim13. Furthermore, overexpression of TbTim13 disrupts interaction between TbTim10 and TbTim17, indicating a greater affinity of TbTim13 for TbTim17. RNAi studies indicated that among all small TbTims, TbTim13 is most crucial to maintain the steady state levels of TbTim17 and the TbTIM17 complex. Analysis of the small TbTim complexes by BN-PAGE and size-exclusion chromatography revealed that TbTim9, TbTim8/13, and TbTim10 form the core structure, whereas TbTim10 in this complex can be replaced by either TbTim12 or TbTim13. Altogether, our results demonstrated that small TbTims are present in more than one complexes and TbTim13 plays a critical role for TbTIM17 complex biogenesis/stability. Therefore, relative to other eukaryotes *T. brucei* possesses a unique architecture of the small TbTim complexes, which could potentially be utilized for designing novel chemotherapeutics.

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Abstract 2703

Elucidating the Mechanism of MBD Protein LLPS and its Role in Heterochromatin Formation and Transcriptional Repression

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Alaji Bah

Membraneless organelles (MLOs) self-assemble into condensed, biochemically distinct microenvironments through liquid-liquid phase separation (LLPS). Heterochromatin, most recently considered an MLO, assembles through weak, multivalent interactions with its associated proteins that contain intrinsically disordered regions. However, the details of the complex molecular interactions between heterochromatin-associated proteins and methylated DNA that drive LLPS have not been fully explored. It is crucial that we elucidate the molecular mechanisms involved in this process as it regulates vital nuclear processes such as transcriptional repression. Its dysregulation is implicated in neurological disorders and cancer. Here, we focus on two members of the methyl-CpG-binding domain (MBD) family of proteins, MBD2 and MBD3, that interpret methylated residues on heterochromatin's underlying DNA. We utilize biochemical and biophysical techniques to (1) determine the conditions and properties that promote MBD2 and MBD3 LLPS and elucidate the molecular mechanism(s) that underpin this process and (2) identify the role binding partners have on MBD2 and MBD3 LLPS. LLPS droplet formation is monitored using UV-Vis spectroscopy and differential interference contrast (DIC) and fluorescence microscopy. To better understand the molecular basis that drives LLPS, small-angle X-ray scattering (SAXS) and dynamic light scattering (DLS) is used to obtain structural and dynamic details of MBD2 and MBD3. Our results provide details into the mechanism(s) by which MBD2 and MBD3 undergo LLPS individually and how this process is enhanced by binding to each other and methylated DNA. Uncovering the driving forces that assemble MBD protein-based LLPS droplets will give us insight into the higher-order organization of heterochromatin and how it functions within this structure. Additionally, understanding how disease-related mutations lead to aberrant formation of condensates or the inability to form condensates will provide novel therapeutic targets.

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Abstract 2721

Targeting of the Tail-Anchored Rab GAP (GTPase Accelerating Protein) Gyp8 to Peroxisomes Is Regulated by the AAA ATPase Msp1

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Monique Quinn, Ariel Lin, Adrian Mora, Chau Vuong, Jenna Maddox, Sean Connolly

Rab GTPase signaling proteins (Rabs) are key regulators of vesicular transport, controlling where and when membranes dock and fuse, ensuring lipid and protein cargoes are delivered to appropriate destinations. Rabs depend upon GTPase accelerating proteins (GAPs) to trigger GTP hydrolysis and return the signaling active, GTP-bound Rab to its inactive, GDP-bound state. Rab GAPs contribute to the efficiency and fidelity of transport pathways, and defective Rab GAPs are implicated in a variety of human diseases. Rab GAPs tend to be cytosolic proteins that transiently localize at membranes to survey for their client Rab. Since its initial discovery, the cellular localization and functions of the yeast (*S. cerevisiae*) Rab GAP Gyp8 have remained essentially uncharacterized. We report that Gyp8 is an atypical transmembrane (TM) GAP whose association with peroxisomes and mitochondria is regulated by the AAA ATPase Msp1, a chaperone that functions to remove tail-anchored proteins from peroxisomes and mitochondria. Computational analysis of Gyp8 predicted a single-pass TM domain near the carboxy terminus, characteristic of a tail-anchored (TA) protein. Subcellular fractionation demonstrated that Gyp8 localized exclusively to a membrane fraction and was resistant to chemical membrane extraction. Fluorescence microscopy indicated that GFP-tagged Gyp8 co-localized with peroxisome, endoplasmic reticulum (ER) and mitochondrion markers in wild type cells. In the absence of peroxisomes, GFP-Gyp8 redistributed to the ER. Loss of machineries regulating membrane insertion or extraction of TA proteins resulted in redistribution of GFP-Gyp8 to mitochondrial membranes. Truncation analyses of Gyp8 indicated that the TM and luminal domains are necessary and sufficient to direct localization to peroxisomes. Growth experiments of cells lacking specific Rab GAPs indicate a peroxisome-related carbon utilization defect in cells lacking Gyp8. Many Rab GAPs enforce spatiotemporal boundraries for activities of their cognate Rab GTPases. We report that Gyp8 spatially restricts localization of the secretory Rab Ypt1. Our results demonstrate regulatory mechanisms to control localization of a transmembrane Rab GAP at multiple organelles, a novel alternative to strategies of transient membrane association and dissociation used by soluble Rab GAPs.

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Abstract 2750

Discovery and characterization of metal-accumulating organelles in bacteria

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Carly Grant, Matthieu Amor, Hector Trujillo, Sunaya Krishnapura, Anthony Iavarone, Arash Komeili

Compartmentalization in the form of protein- and lipid-bounded organelles is a widespread phenomenon in the bacterial world. The study of bacterial organelles can have profound impacts on our view of cellular evolution, reveal novel physiological pathways and provide tools for design of biomedical applications. Here, I will describe our efforts to use a "reverse cell biological" approach to define the genetic blueprint for a widespread class of bacterial lipid-bounded organelle. Electron microscopic imaging of various bacterial species had revealed the presence of unidentified iron and phosphorus rich compartments which we have named ferrosomes. We identified the proteome of ferrosomes purified from Desulfovibrio magneticus RS-1 using mass spectrometry. We then developed a genetic system for RS-1 and showed that the ferrosome (or fez) genes are necessary for ferrosome formation. We found that at least 17 different phyla of bacteria and archaea contain putative fez gene clusters. Multiple bacteria with fez gene clusters can form ferrosomes and mutants lacking ferrosomes show delayed growth under extreme iron starvation conditions. Furthermore, the fez cluster is sufficient for ferrosome formation when expressed in heterologous hosts such as *E. coli* and other bacterial species. Altogether, this work defines a new class of bacterial lipid-bounded organelles and an unrecognized mode of iron homeostasis. We now aim to understand the chemical composition of ferrosomes, define the functions of each ferrosome gene, and understand the physiological integration of ferrosomes within diverse bacterial species.

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Topic Category Protein Synthesis, Structure, Modifications and Interactions

Abstract 152

Discovery of a Mammalian Amyloid Disaggregase

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The ability of cells to adapt to a wide variety of stress conditions plays a critical role in various physiological and pathological settings, including development, cancer and neurological disorders. We recently reported the discovery of stress-induced low complexity noncoding RNA derived from stimuli-specific loci of the ribosomal intergenic spacer (rIGSRNA); an enigmatic region of the human genome historically dismissed as "junk" DNA. We showed that low complexity rIGSRNA activate a physiological amyloidogenic program that converts the nucleolus into Amyloid-bodies: a molecular prison of immobilized proteins in an amyloid-like state. This conserved post-translational regulatory pathway enables cells to rapidly and reversibly store an array of endogenous proteins in Amyloid-bodies and enter a dormant-like phenotype in response to severe environmental insults. While many membrane-less compartments have been described as liquid-like (e.g., stress granules, P-bodies, germ cell granules), our discovery of Amyloid-bodies provided evidence of an amyloidogenic process that can physiologically transition biological matter to a solid-like state. The ability of mammalian cells to efficiently dissemble Amyloid-bodies raises a fundamental question: Are mammalian cell disaggregases involved in Amyloid-body disassembly? Here, we show that Amyloid-bodies undergo a solid-to-liquid-phase transition to release sequestered proteins and restore nucleolar functions. An RNAi screened identified key Heat Shock Proteins (Hsps) that remodel Amyloid-bodies back to the liquid nucleoli on stress termination. The composition of mammalian disaggregases differs considerably from non-metazoans and those involved in disassembly of pathological amyloids *in vitro*. Activation of mammalian disaggregases and Amyloid-body disassembly is dependent on ATP concentration. Conceptually, this work identifies metazoan disaggregases challenging the widely accepted paradigm that the amyloid state is irreversible in mammalian cells. The data also provides alternative insights into pathogenic amyloids by examining their disassembly in cellular systems.

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Abstract 153

Examination of the affinity of anti-SARS-CoV-2 IgA, which is mucosal immunity, for SARS-CoV-2 BA.4 variant and BA.5 variant

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Currently, the variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is the mainstream of COVID-19 in the world, are the omicron type SARS-CoV-2 BA.4 variant and BA.5 variant derived from the omicron variant. In Japan, the Pfizer/BioNTech COVID-19 vaccine (BNT162b2) and the Moderna COVID-19 mRNA-1273 vaccine, which were created based on the viral gene of the Wuhan-type SARS-CoV-2 B.1.1.7 variant, have been inoculated. Previous clinical studies have reported that vaccination with these mRNA-based COVID-19 vaccines secretes a large amount of anti-SARS-CoV-2 IgA, which is mucosal immunity, into breast milk. However, recent clinical studies revealed that SARS-CoV-2 BA.4 and BA.5 variants have possessed the ability to successfully circumvent anti-SARS-CoV-2 immune mechanisms conferred by COVID-19 vaccination or prior SARS-CoV-2 infection. Therefore, we investigated the affinity of anti-SARS-CoV-2 IgA (PDB:CV2.1169 and PDB: CR3022) against SARS-CoV-2 B.1.1.7 variant (PDB: 7QEZ_A), SARS-CoV-2 BA.4 variant and BA.5 variant (PDB: 7XNS) by in silico analysis. As a result of the in silico analysis, the affinity of IgA CV2.1169 for each SARS-CoV-2 variant (B.1.1.7 variant, BA.4 variant, BA.5 variant) was −15.92 Kcal/mol, −9.26 Kcal/mol, −8.94 Kcal/mol. The affinities of IgA CR3022 for each SARS-CoV-2 variant (B.1.1.7 variant, BA.4 variant, BA.5 variant) were −16.35 Kcal/mol, −9.85 Kcal/mol, and −9.24 Kcal/mol. In addition, IgA derived from individuals vaccinated with the Pfizer/BioNTech COVID-19 vaccine (BNT162b2) or the Moderna COVID-19 mRNA-1273 vaccine was found to have no high affinity to the omicron type SARS-CoV-2 BA.4 and BA.5 variants. In other words, the anti-SARS-CoV-2 IgA (PDB: CV2.1169 and PDB: CR3022), which has strong binding power to Wuhan type SARS-CoV-2 B.1.1.7 variant, was found not to have a strong affinity for omicron type SARS-CoV- 2 BA.4 variant and the BA.5 variant. In Japan, it has been reported that the COVID-19 vaccine for omicron type SARS-CoV-2 BA.4 variant and BA.5 variant may be approved in November 2022. We hope that the new COVID-19 vaccine will become widespread.

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Abstract 1169

Elucidating binding modes of hnRNP H to RNA targets using 19F NMR paramagnetic relaxation enhancement

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Dynamic protein-RNA interactions play an essential role in helping RNA-binding proteins (RBPs) find the correct binding sites in an RNA that may contain multiple copies of its binding motif. Unfortunately, these interactions are difficult to study since, by definition, they are dynamic. Heterogeneous nuclear ribonucleoprotein (hnRNP) H is a dynamic RBP that participates in many stages of RNA metabolism by binding to G-tract RNA sequences. It has two quasi-RNA-recognition motifs (qRRMs) connected by a flexible linker that are posited to work in tandem to bind in a closed conformation to one G-tract or bind in an open conformation to two G-tracts. However, it is currently unknown under what conditions an open conformation is preferred over a closed one. Presented herein is a proof-of-concept method by which fluorine-19 (19F) NMR paramagnetic relaxation enhancement (PRE) can be utilized to evaluate these conditions. 19F NMR is a useful one-dimensional NMR approach to studying RNA binding because 19F is the only stable isotope of fluorine, and it does not naturally occur in proteins. Therefore, a fluorine probe like 3-bromo-1,1,1-trifluoroacetone (BTFA) can be attached to the protein in a site-specific manner and serve as a reporter. Similarly, a nitroxide free-radical spin label like IAM-PROXYL can be attached to phosphorothioated nucleic acid binding target in order to measure intermolecular PREs. A preliminary set of short, synthetic DNA oligomers containing either a 5′ G-tract, a 3′ G-tract, or both was designed with the paramagnetic spin-label site on the 5′ end. A duplicate set was designed with a spin-label site on the 3′ end. Each oligomer was then titrated into 19F-labelled hnRNP H, and the corresponding 19F NMR spectra were collected. The same was done for the diamagnetic forms of the oligomers. Two conclusions can be drawn from the resulting spectra: 1.) these systems provide a proof of concept that 19F NMR PREs can reveal both qualitative and quantitative information regarding hnRNP H – G-tract binding mechanisms, and 2.) hnRNP H exhibits a preference for one G-tract over the other.

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Abstract 1173

Mechanisms regulating the specificity of NCK1/2 adaptor proteins

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Signaling pathways downstream of receptor tyrosine kinases (RTKs) are essential for organogenesis and cell homeostasis. RTK signaling is often relayed via adaptor proteins that recruit target proteins to form signaling complexes. Homologous adaptor proteins NCK1 and NCK2 are composed of a single SRC Homology (SH) 2 interaction domain that binds phosphorylated tyrosine-containing motifs, and three SH3 domains that are capable of binding proteins containing poly-proline motifs. Although NCK1 and NCK2 are often considered redundant, we have previously demonstrated via proteomic approaches that each may associate specifically with a subset of their protein targets. However, the molecular mechanisms underlying interaction specificity are still unresolved. We hypothesized that the disordered regions between SH2-SH3 domains (i.e. interdomain regions) of NCK1 and NCK2 proteins modulate their specificity. Using an affinity purification approach combined with mass spectrometry (AP-MS), we compared the interactome of NCK1/2 WT proteins to that of chimeras for which the interdomain regions are inverted (i.e. NCK1(NCK2i1,i2,i3) and NCK2(NCK1:i1i2i3)). We observed that 22 'NCK2-specific' proteins may also bind NCK1(NCK2:i1, i2,i3), despite the presence of NCK1's own SH2/3 s. We confirmed this change in specificity for candidates ARHGEF10L and PSTPIP2 using pull-down assays. We also found that, in addition to the interdomain regions, the SH2 domain may play a central role in mediating target binding specificity. Our work will explain how simple adaptor proteins may transduce specific signals from the same RTK to establish distinct cellular phenotypes.

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Abstract 1191

Adhesion and phase behavior of intrinsically disordered peptides from bacterial biofilms

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Adhesives that function in wet environments are widely used in ships, fishing industry, etc. Also, bio-adhesives are extensively used in biomedical applications where there is a need to adhere two wet surfaces. To search for better adhesive materials that function in an aqueous environment, engineers have extensively studied adhesive proteins from mussels and barnacles, leading to major insights into biological adhesion, often involving intrinsically disordered domains that can form phase separated condensates. However, mussel foot proteins (mfps) are sensitive to environmental conditions including oxygen and pH, limiting their wide application. In a serendipitous discovery while studying how *Vibrio cholerae* biofilms adhere to surfaces, we discovered a short peptide sequence made of 57-amino acids that is majorly responsible for Vc adhesion to various abiotic surfaces. Abundant in lysine, tyrosine, tryptophane, and threonine, the sequence shares similarity with mfps but also important differences. In this talk, I am going to describe our recent progress in understanding how this unique sequence balances the tendency to LLPS and to adhere to surfaces in order to maximize its adhesive performance.

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Abstract 1219

Development of Expression and Purification Protocols for Characterizing Members of the Ly6 Protein

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The Ly6 proteins are a family of novel regulatory proteins conserved among multiple species that share significant structural homology with alpha neurotoxins commonly found in snake venoms, such as α-bungarotoxin. Previous work studying Ly6 function has relied on *in vivo* monitoring of endogenous protein levels/behaviors; however, the ability to biochemically characterize and study Ly6 function using purified protein has been limited. Ly6 proteins share a conserved motif known as the Ly-6/uPAR (LU) domain, in which 10 cysteines form five disulphide bonds that maintain the highly flexible three-fingered structure of the LU domain. These predicted structural elements make the expression of Ly6 proteins in their soluble form challenging. In this study, optimal recombinant expression in *E. coli* and purification conditions for the ODR-2, a membrane-associated protein related to the Ly6 superfamily of GPI-linked signaling proteins found in *C. elegans*, were elucidated. ODR-2 has two predicted signal sequences, an N-terminal signal sequence for export to the plasma membrane and a C-terminal signal sequence for GPI attachment. We chose to test expression for constructs that were full length or had both signal sequences removed (to mimic the predicted sequence for the mature protein). We examined the impact of using a variety of solubility tags to support expression of soluble ODR-2; we found that use of N-terminal GST, MBP, and pelB leader sequence tags did not improve soluble expression while an N-terminal SUMO-tag did promote soluble expression in the supernatant. All constructs showed significant insoluble expression in the pellet. These conclusions were consistent across multiple expression strains, including BL21 DE3, Origami-2, Rosetta-gami, and SHuffle. Surprisingly, we found that optimal expression yield occurred at 30 or 35°C and a 3-hour inoculation time. We are in the process of scaling up expression and developing protocols for purification of both soluble and insoluble ODR-2.

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Abstract 1229

Pro-apoptosis protein Bax is regulated by the ubiquitin-proteasome system

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Hae-Seul Choi, Soo-Yeon Kim

Bcl-2 family proteins are closely related to apoptosis. Among them, Bax is well known as a pro-apoptotic protein. It is an inducer of apoptosis at the mitochondrial level. The inactive form of Bax is located in the cytosol. But, the active form of Bax is translocated to the outer membrane of mitochondria. Then, Bax forms an oligomerization with Bax or Bak which increases mitochondrial outer membrane permeabilization (MOMP) and cytochrome c is released from mitochondria. Finally, caspases are activated by these responses and induce apoptosis. The purpose of this study was to investigate whether Bax is regulated by the ubiquitin-proteasome system. In the current study, we ascertained the presence of deubiquitinating enzymes (DUBs) associated with Bax by performing the yeast two-hybrid screening (Y2H). We determined that two ubiquitin-specific proteases (DUB-1 and DUB-2) are associated with Bax. Through immunoprecipitation and GST pull-down assays, we found that these DUBs directly interact with Bax. Binding of these DUBs to Bax show interaction as a deubiquitinating enzyme (DUB), which regulates ubiquitination on Bax. The enzyme assay revealed that these DUBs have deubiquitinating activity by detaching ubiquitins from Lys63-linked chains, indicating that they affect the cellular functions of Bax including the regulation of stress signals such as UV damage, hypoxia, oxidative stress, and DNA damage, but they are not related with proteasomal degradation. In addition, the half-life of the Bax protein was also determined by performing site-directed mutagenesis of putative ubiquitination sites on Bax. Of these, two lysine sites in Bax showed less ubiquitination, resulting in a longer half-life compared to wild-type Bax.

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Abstract 1230

Molecular Mechanisms of Holliday Junction Branch Migration Catalyzed by an Asymmetric RuvB Hexamer

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The Holliday junction (HJ) is a universal DNA intermediate of homologous recombination that is involved in many fundamental physiological processes. In bacteria, RuvB, a motor protein of the AAA+ ATPase superfamily, drives branch migration of the Holliday junction with a mechanism that had yet to be elucidated. We determined two cryo-EM structures of RuvB in complex with DNA and nucleotides, providing a comprehensive understanding of HJ branch migration. Six RuvB protomers assemble into a spiral staircase, in the shape of a ring, with DNA in the central pore. Four protomers of RuvB hexamer interact with the backbone of the DNA substrate, suggesting a pulling-and-revolving mechanism of DNA translocation with a basic step size of 2 nucleotides. Moreover, the variation of nucleotide binding states in our RuvB hexamer supports a sequential model for ATP hydrolysis, ADP release, and ATP reloading, which occur at specific positions on the RuvB hexamer. Furthermore, the asymmetric assembly of RuvB also explains the 6:4 stoichiometry between RuvB and RuvA, which assembles into a complex to coordinate HJ migration in cells. Taken together, we provide a comprehensive framework for the mechanistic understanding of HJ branch migration facilitated by RuvB motor protein, which may be universally shared in both prokaryotic and eukaryotic organisms.

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Abstract 1232

Investigating the Mechanism of CowN Protection of Nitrogenase Against CO Inhibition

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Dustin Willard, Cedric Owens

Fixed nitrogen enters the biosphere through two major pathways: the industrial Haber Bosch process that is used to make fertilizer and biological nitrogen fixation by the enzyme nitrogenase. A major drawback of the Haber Bosch process is that it requires high temperatures, high pressure, and natural gas, thereby causing environmental pollution. Nitrogenase, in contrast, converts nitrogen gas into ammonia under ambient conditions using biological energy in the form of ATP, and is thus a more environmentally friendly nitrogen fixation process. If we were able to increase the usage of biological nitrogen fixation, the need for fertilizers would decline. Nitrogenase is inhibited by carbon monoxide (CO), meaning that when CO is present, nitrogen fixation will not occur. CO is a naturally occurring gas so bacteria containing nitrogenase must find a way to avoid inhibition. It was discovered that nitrogenase is protected by another protein, CowN. CowN permits nitrogenase activity to persist in the presence of CO by forming protein-protein interactions with nitrogenase. This presentation describes our recent work to identify how CowN and nitrogenase interact. A conserved C-terminal Glu87 residue on CowN is necessary for full protection against CO inhibition as Glu87Ala, Glu87Gln, and Glu87Asp variants impair CowN protection. Interestingly, mutations at other conserved C-terminal residues do not affect CowN function. Glu87 mutations decrease CowN's ability to bind to nitrogenase, suggesting that Glu87 forms a salt bridge with nitrogenase. Mass spectrometry analysis of crosslinked Cown-MoFeP complexes identified several potential interaction sites on nitrogenase, notably one located near a proposed gas channel and another at its active site. Together, this work suggests that CowN interacts with nitrogenase through an important C-terminal salt bridge and may bind to nitrogenase in more than one location.

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Abstract 1233

In vitro reconstitution of ribosome biogenesis

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Background: Ribosome biogenesis is a recursive process in which nascent ribosomes are synthesized by pre-existing ribosomes and is an essential process for the self-replication of life. Hence, reconstituting ribosome biogenesis *in vitro* is crucial to the understanding of the self-replication of life. However, this goal has not been achieved due to its complexity. In this study, we tried to achieve the first successful reconstitution of the ribosome biogenesis *in vitro*.

Method: To prove the reconstitution of ribosome biogenesis *in vitro*, we developed a highly specific and sensitive assay to detect nascent artificial ribosomes. For the specific detection, we utilized orthogonal translation. The efficiency of translation initiation is mainly determined by the interaction of the Shine–Dalgarno (SD) sequence on mRNAs and the anti-SD (ASD) sequences on ribosomes. Therefore, modified SDs and ASDs (orthogonal SD/ASD; oSD/oASD) lead to the orthogonal translation in which ribosomes with oASD specifically translate mRNA with oSD. We screened oSD/ oASD pairs which were reported to show orthogonality *in vivo* to determine oSD/oASD pairs which show orthogonality *in vitro*. For the highly sensitive detection, we developed a femtoliter droplet assay. In this assay, a slight amount of reaction solution is confined to femtoliter droplets, which results in highly sensitive enzymatic activity detection. Furthermore, we developed a deep-learning-assisted analysis pipeline that enables accurate and automated evaluation of the ribosome activity in each droplet. Finally, we tackled to reconstitute *E. coli* ribosome biogenesis *in vitro* by co-expressing the rRNA operon gene and 54 ribosomal protein (r-protein) genes in a cell-free transcription and translation (cell-free TXTL) system based on an optimized *E. coli* S150 extract mimicking cytoplasmic conditions.

Results and Discussion: To develop a specific assay, we screened seven oSD/oASD pairs that showed orthogonality in *E. coli*. As a result, we found that the or1-oSD/oASD pair enables specific detection of orthogonal ribosomes *in vitro*. To develop a highly-sensitive assay, we optimized cell-free TXTL in femtoliter droplets and enabled the detection of single ribosomes in femtoliter droplets. Using the highly specific and sensitive assay, we tried to reconstitute ribosome biogenesis. First, we explored reaction conditions using simplex-lattice design and optimized the concentration of the native ribosomes, rRNA operon, and 21 r-protein genes which constitute the small subunit (SSU) of ribosomes. As a result, we detected a translational signal derived from SSU biogenesis *in vitro*. Next, we optimized the concentration of the rRNA operon gene and 33 r-protein genes which constitute the large subunit (LSU) of ribosomes, which also result in the successful reconstitution of LSU biogenesis *in vitro*. Finally, we co-expressed all of the ribosomal component genes including the rRNA operon gene and 54 r-protein genes at the optimal condition of LSU biogenesis *in vitro* and confirmed the synthesis of SSU and LSU in a single reaction. Also, we detected and quantified 54 nascent r-protein by mass spectrometric analysis. This study achieved the first successful reconstitution of ribosome biogenesis *in vitro*, which will facilitate the understanding of the self-replication of life, the creation of self-replicating artificial cells, and designing artificial ribosomes with altered functionalities.

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Abstract 1236

The vasoprotective transcription factor Grainyhead-like 3 exerts extra-nuclear functions and is regulated by plant-derived agents in young and senescent endothelial cells

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Vascular aging is accompanied by a decline in vessel functionality. Hallmarks of this functional impairment are a decreased bioavailability of nitric oxide (NO) by reduced expression of endothelial nitric oxide synthase (eNOS) resulting in impaired migratory capacity and increased apoptosis sensitivity of endothelial cells (EC). Furthermore, this goes along with a disturbed redox balance and decreased mitochondrial functionality, which all contribute to induction of cellular senescence in EC, being an important aspect of endothelial dysfunction. Caffeine and curcumin, two plant derived agents, are known to act vasoprotective. Moreover, we have shown that the transcription factor Grainyhead-like 3 (GRHL3) also improves vascular functionality. It is expressed in EC and increases both NO bioavailability and migratory capacity, while reducing apoptosis sensitivity. However, the regulation of GRHL3 in cellular senescence as well as by caffeine and curcumin have not been investigated yet. When studying the mechanisms by which GRHL3 increases vascular functionality, we showed in en face preparations of arteries from different vascular beds of mice that GRHL3 also localizes outside of the nucleus in myo-endothelial projections, membrane protrusions of EC which are in direct contact with the adjacent smooth muscle cells. Thus, we hypothesized that GRHL3 exerts extra-nuclear functions. To test this assumption, we generated a GRHL3 mutant that can no longer enter the nucleus and interestingly, found that this protein can still exert vasoprotective effects by inhibiting EC apoptosis and by improving migratory capacity and NO bioavailability. Using a proximity ligation assay, we found that GRHL3 interacts with eNOS *ex vivo* and in arteries of mice *in vivo*, which could serve as an explanation for its effects on NO-bioavailability. To investigate the role of GRHL3 in cellular senescence and the effects of caffeine and curcumin on both, we first determined whether they affect GRHL3 expression per se. A 24 h incubation of EC with either substance led to elevated GRHL3 levels. To induce senescence, EC were treated with low dose H2O2 every second day for two weeks. This resulted in an increase in senescence markers like the cell cycle inhibitor p21, as well as a decrease in eNOS. Interestingly, also GRHL3 levels were reduced in the senescent cells. Coincubation with caffeine attenuated senescence induction and maintained GRHL3 levels. In conclusion, we could show that GRHL3 not only acts as a transcription factor in the nucleus, but also has important extra-nuclear functions in EC. Furthermore, caffeine and curcumin positively affect GRHL3 levels, and caffeine even after senescence induction. It will be interesting to determine if GRHL3 plays a causal role in protection against cellular senescence, and thus, if approaches aimed at sustaining or increasing GRHL3 levels could have therapeutic value in delaying vascular aging and associated pathologies.

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Abstract 1256

The surface-specific antigenic peptide designs of HPIV-type 2 Hemagglutinin-Neuraminidase and Fusion protein for anti-viral immunotherapy drug development

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Juhyeong Lee, Kay Perry

Background Human parainfluenza viruses (HPIV) are known to be highly contagious viruses that cause severe upper and lower respiratory tract infections. In particular, HPIV type 2 accounts for 60% of all infections. This transmissibility is achieved by cell-to-cell fusion mediated by the Hemagglutinin-Neuraminidase (HN) and Fusion (F) protein that form a complex by dynamic structural changes with the conformational change of the HN head domain and F protein-cleavage during penetrating cells. *In vivo*/*in vitro*, the commercial antibodies that are able to bind to these viral proteins have shown a very low detectable yield with limited success. It is urgent to identify the antigenic sequences of two proteins for accurate viral molecular diagnosis and development of therapeutic agents. Methods The predicted models of unknown structures of HPIV type 2 HN and F proteins are valuable to try a better understanding of secondary structure, tertiary folding and interface region with other proteins. We predicted the structures of the full lengths HPIV type 2 Hemagglutinin-Neuraminidase (M1-P571) and Fusion protein (M1-S551) through carefully homology modeling (the search for templates is based on searching PDB profile database, running BLAST, extracting distance constraints from 121 templates, structurally superpose, and predicting oligomeric state conservation). The PDB 1Z4 V (PIV 5; identity 49%, homotetramer), 5B2D (Mumps virus; identity 44%, homodimer), 4fzh (Newcastle virus; identity 36%, homotetramer) for HPIV HN and PDB 2b9b (PIV 5; identity 52%, homotrimer), 3maw (Newcastle virus; identity 35%, homotrimer), 5evm (Nipah virus; 27% identity, homotrimer) for HPIV F protein, respectively. To predict the complex (protein-protein interaction) interface between two proteins, we focused on the potential structural movement (a flexible loop between Hn' head and stalk with sialic acid binding) and cleavage at the surface regions using various docking model servers and tools (AutoDOCK, ClusPro, PatchDock, Firedock, Dcomplex server and MolFit). Furthermore, we designed the potential antibody-binding regions with molecular simulation of a dynamic protein complex to observe the transition status at average time intervals of 50–100 ns. Results The conformational change between active form (binding status)/inactive form (non-binding status) of HN induces cell fusion activation by 4-head up (with sialic acid binding) or inactivation by 4-head down (without sialic acid binding) depending upon a flexible loop (L100-N130). The 4 helix bundles of stalk regions (1M-N120) may be sufficient for complex with F protein and F-interacting domain that is hidden before binding is located between I80-L100. The interface is weak and transient due to F protein' conformational change (the computational-aided mutations of interface induces a lower fusion activity). The best surface antigenic domains are positioned at N355-Y369 (-NDPEPTSQNALNPNY-) or G329-C343 (-GVMPCNATSFCPANC-) for HN and at T98-L114 (-TDTKTRQKRFAGVVVGL-) or A392-G405 (-ADPPHVVSQDDTQG-). These results revealed the potential antigenic sequences for therapeutic antibody or drug agents through the predicted binding interface of Hn-F complex between active-inactive forms. Conclusion The sequences to be considered as antigenic peptides through the predicted complex structures with conformational changes of the HN-F protein are identified and presented to get better understanding the mechanism of antibody development or therapeutic agents.

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Abstract 1259

TRIM33 as a novel reader of metabolically-derived histone lactylation

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Brian Smith

Macrophages are ubiquitous in all major tissues, and their essential role in inflammation is linked to the development and progression of a wide variety of inflammatory diseases. Specifically, macrophage activation is crucial in mediating inflammation. Transition from inflammatory (M1) to reparative (M2) macrophage polarization is critical in regulating the inflammatory response. This transition was recently shown to be mediated by the glycolytic metabolite, lactate, through a novel metabolically-derived histone post-translational modification (PTM), lysine lactylation (Kla); however, the mechanism and critical protein players tying histone Kla to the macrophage polarization transition are unknown. Here, we identified proteins that recognize and bind sites of histone Kla, providing a link between histone Kla and macrophage transition. We focused on bromodomains due to their well-established ability to bind histone lysine acetylation (Kac) which is chemically similar to Kla and their role in transcriptional regulation in the context of larger proteins. We performed a screen with our custom synthesized library of histone peptides containing distinct histone Kla sites against recombinantly-expressed bromodomains using the AlphaScreen assay, a luminescent proximity bead-based assay. We found that TRIM33 was the only bromodomain in our screen that bound sites of histone Kla. TRIM33 regulates hematopoiesis, macrophage polarization, and the recruitment of myeloid cells to sites of inflammation. In particular, TRIM33 is essential for the transition from M1 to M2 macrophages, making TRIM33 an intriguing potential link between histone Kla and macrophage polarization transition. We then used orthogonal biophysical techniques, including Isothermal Titration Calorimetry (ITC) and 2D protein-detected nuclear magnetic resonance (NMR), to validate and determine the binding affinity of the TRIM33 bromodomain towards sites of histone Kla compared to Kac. In addition to the bromodomain, the adjacent plant homeodomain (PHD) was required for high-affinity TRIM33 binding to acylated histone peptides. Multiple sequence alignments including all 61 human bromodomains revealed a residue within the binding pocket that is unique to TRIM33 that may mechanistically explain its specificity for Kla compared to the other bromodomains. Thus, our studies implicate TRIM33, a bromodomain-containing protein, as a novel binder of histone Kla and the potential missing link between histone Kla and macrophage polarization.

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Abstract 1267

The Role of Hsp90-R2TP in Ciliogenesis

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Cilia are microtubule-based structures that act as sensory organelles (primary cilia) or drive locomotion (motile cilia). Defects in the development and function of cilia have been linked to several human genetic diseases and certain cancers. Deleted in primary ciliary dyskinesia (DPCD) is a gene encoding a 23 kDa protein of unknown function and structure that has been implicated in motile and nodal ciliogenesis. We identified DPCD as a new component of the R2TP chaperone complex. R2TP is a highly conserved chaperone complex formed by two AAA+ ATPases, RUVBL1 and RUVBL2, that associate with PIH1D1 and RPAP3 proteins. RUVBL1/2 form a hexameric complex. PIH1D1 contains PIH1 domain and a CS domain, while RPAP3 contains two TPR domains and a domain interacting with RUVBL2. R2TP functions with several other chaperones including Hsp90 and Hsp70 to promote macromolecular complex formation. We found that Hsp90-R2TP-DPCD act to regulate ciliogenesis through modulating signaling pathways. Our findings highlight a new function for Hsp90-R2TP in maintaining cellular protein homeostasis.

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ASBMB

Abstract 1275

Probing Anion-Protein Interactions Beyond the Hofmeister Effect: Biophysical and in silico Characterization of Prokaryotic Nitrate Binding Proteins

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Elizabeth Pack, Caden Maydew, Kevin Alberto, Steven Nielsen, Gabriele Meloni, Sheel Dodani

Anions play important roles in all forms of life. Nature has evolved proteins as supramolecular hosts to recognize, translocate, and transform anions for a range of biological functions. These functions can be achieved with exquisite selectivity, despite similarities in anion size, shape, and charge. To elucidate the supramolecular principles that govern selective anion-protein receptor interactions beyond the classic Hofmeister phenomena, we have developed a hybrid experimental and theoretical workflow. In this presentation, I will describe our investigation into the coordination plasticity of soluble prokaryotic nitrate binding proteins. We have dissected the thermodynamic and kinetic contributions of anion binding with isothermal titration calorimetry and stopped-flow fluorescence. This has been complemented with molecular dynamic simulations to reveal how global protein motions compensate to accommodate the binding different anions. Taken together, our findings lay the foundation to establish new methods and paradigms to study anion-protein interactions.

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Abstract 1277

SUMOylation "hot spots" in the piRNA pathway and heterochromatin revealed by diGly proteomics

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Katalin Fejes-Toth, Alexei Aravin

Protein modification by the small ubiquitin-like modifier SUMO is involved in a myriad of essential processes, including chromatin organization and regulation. However, despite its critical functions, the specific targets and mechanistic implications of SUMOylation in different pathways are still poorly understood, partly owing to the often transient nature and technically challenging detection of this modification. Previous work showed that SUMO pathway deficiencies in the Drosophila female germline cause severe gene deregulation, transposon activation, and sterility. To further our understanding of the mechanistic role of SUMO, we developed a transgenic model and diGly proteomics-based strategy for unbiased characterization of the ovarian 'SUMOylome' with aminoacid-level resolution. This approach uncovered a high-confidence set of SUMO sites in several hundred proteins. Strikingly, we found that the most overrepresented functional categories of SUMO targets include proteins associated with heterochromatin regulation and the piRNA pathway – the primary pathway that enforces post-transcriptional and transcriptional silencing of transposons in animal germlines. Furthermore, we found that several proteins involved in distinct aspects of piRNA biogenesis and function are multi-SUMOylated in a manner that depends on the central piRNA effector Piwi, indicating a multifaceted role of this modification in active transposon response. Together, these data highlight unexpectedly broad and diverse implications of protein SUMOylation in heterochromatin regulation and the cellular defense against genomic parasites.

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Abstract 1288

Analyzing F1-ATPase to Determine Elasticity of the γ-Subunit Rotary Shaft and its Influence on Chemomechanical Movement

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Kaitlin Snodgrass

F_1-ATPase is the enzyme in cells which produces ATP, yet despite its importance to the survival of living things, its exact behavioral patterns remain unclear. The species *Paracoccus Denitrificans* F_1-ATPase follows a 120° rotationally pseudo symmetric movement pattern, categorized by a series of transition and dwell states, during which the enzyme movement will fluctuate due to its natural elasticity and the influence of Brownian motion. Previous single molecule imaging experiments have revealed a relationship between the viscoelastic nature of the shaft and the rotational speed and energy efficiency of its movement. Virtual simulation programs such as VMD can be used to observe overall protein structure and isolate relevant protein domains. The goal is to display the elastic nature of the shaft and understand the reason for its behavior. Molecular dynamic simulations can be used to map the locations of the individual molecules contained in the γ-subunit rotary shaft and track their movement as the enzyme rotates. Recent single molecule spectroscopy developments have been able to provide experimental data for analysis. Through various methods such as extracting the rotational velocities from the recorded positions, it is possible to view the rate and rotational speed at which the enzyme fluctuates during the time of a dwell. By looking at the data acquired from both virtual simulations and physical experiments, light can be shed on the elastic properties of the γ-subunit. In doing so, a new aspect of the F_1-ATPase's behavior previously unknown may be elucidated, moving one step closer to fully understanding and possibly manipulating the behavior and ATP production of the enzyme in doing so.

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Abstract 1299

Investigating pH-Dependent Dynamics of β-catenin and Proteostatic Regulatory Mechanisms

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Katharine White

Transient changes in intracellular pH (pHi) are a regulator of cellular processes including metabolism, proliferation, migration, signaling, and apoptosis. These processes are mediated by pH sensors, proteins with pH-dependent activity, conformation, or abundance. One identified pH sensor is β-catenin, a Wnt signal transducer and an adherens junction protein. Prior work showed decreased abundance of β-catenin at high pHi from enhanced proteasomal-mediated degradation mediated by the E3-ligase, β-TrCP. However, the spatial and temporal dynamics of β-catenin have not been studied in the context pHi. Here we show that high and low pHi conditions differentially altered subcellular pools of β-catenin. We found that high pHi resulted in a loss of β-catenin from cell junctions as well as the nucleus and cytoplasm. Conversely, low pHi stabilized nuclear and cytoplasmic β-catenin pools but did not affect abundance at cell junctions. Furthermore, β-catenin (plakoglobin) was significantly enriched at the membrane with high pHi, revealing a potentially novel mechanism for rescuing the loss of junctional-associated β-catenin at high pHi to maintain epithelial cell-cell junctions. Our results demonstrate that cellular pHi dynamics can titrate β-catenin stability and alter subcellular distribution. To investigate the spatiotemporal dynamics of β-catenin under various pHi conditions, we expressed a photoconvertible β-catenin-mMaple3 and performed time-lapse microscopy to monitor re-localization and degradation rates of junctional, cytoplasmic, and nuclear β-catenin with and without pHi manipulation. This approach allows us to explore whether pHi differentially regulates the lifetime of distinct subcellular pools β-catenin. Our combination of biochemical assays and live, single-cell time-lapse microscopy experiments allow us to dissect distinct roles for pHi in regulating the adhesion and transcriptional function of β-catenin. These results have implications for β-catenin in regulating both normal pH-dependent cellular behaviors and pathological behaviors in diseases where pHi is dysregulated. Future work will use proteomics approaches to broadly identify other proteins with pH-dependent abundances across the human proteome.

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Abstract 1307

Investigation of methyl coenzyme M reductase (MCR); enzyme assembly, post-translational modifications and coenzyme F430 delivery

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Today one of the biggest concerns plaguing our society is the issue of global warming. The greenhouse gases that are the largest contributors to global warming include: carbon dioxide (fossil fuel and industrial, 76%), methane (16%), nitrous oxide (6%), and fluorinated gases (2%). Methanogenesis, the process by which methanogenic archaea produce methane, is of considerable interest in current research, specifically due to its environmental implications. MCR, or methyl-coenzyme M reductase, catalyzes the final step of the methanogenic pathway converting methyl-coenzyme M and coenzyme B into methane and a heterodisulfide of coenzyme M and coenzyme B. A homolog of MCR is also found in anaerobic methanotrophic archaea (ANME), which catalyze the anaerobic oxidation of methane (AOM). MCR is only active in the presence of reduced coenzyme F430, a unique nickel-containing tetrahydrocorphin. Within the active site, the alpha subunit of MCR contains distinct post-translationally modified residues. Comparative genomics, enzyme characterization, and biophysical methods are being employed to determine the enzyme(s) responsible for the 1-N-methylhistidine modification and to investigate the delivery mechanism of coenzyme F430 during the assembly of holo MCR. Understanding the roles of each PTM and the enzymes responsible for their biosynthesis is critical for the long-term goal of heterologous expression of active MCR, which could lead to the development of an industrial system that consumes environmental methane and can help mitigate global warming.

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Abstract 1309

Cytosolic quality control triages degradation of mistargeted secretory proteins based on thermodynamic stability

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Khanh Nguyen

Secretory proteins are canonically targeted to the endoplasmic reticulum (ER). Proteins that fail to enter the ER, for example during preemptive quality control, present a threat to cytosolic proteostasis. To protect the cytosol, it is expected that cytosolic quality control machineries will rapidly redirect mistargeted proteins towards degradation. However, this process has been difficult to observe in living cells, where mistargeted protein populations can be small compared to the amount of properly targeted protein. We have demonstrated that genetically encoded peroxidase proximity labeling is an effective approach for quantifying mistargeted secretory proteins in the cytosol. Here, we apply peroxidase proximity labeling in HEK293T cells to characterize how mistargeted secretory proteins are targeted for degradation, using mutants of transthyretin (TTR) and alpha-1-antitrypsin (A1AT). We find that the signal sequence of mistargeted TTR is rapidly proteolyzed to generate an N-terminus that is resistant against N-end rule proteasomal degradation. This persistent species does not form native tetramers, is aggregation-prone, and is slowly cleared through autophagy. Lowering the thermodynamic (but not kinetic) stability of TTR decreases this proteolysis, leading to rapid proteasomal clearance of the intact mistargeted protein and avoiding accumulation of TTR aggregates. Similar behavior is observed for A1AT. Hence, we conclude that the clearance of mistargeted secretory proteins relies upon a quality control process that triages mistargeted proteins based on their thermodynamic stability. Because this quality control mechanism prioritizes degradation of the most destabilized proteins, it counterintuitively leads to greater aggregation of thermodynamically stable TTR variants in the cell.

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Abstract 1316

Phosphorylation of Ribosomal Proteins by p21-Activated Protein Kinase 2 (PAK2) is a novel mechanism to inhibit mRNA translation under oxidative stress

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Lingyun Xu, Zhongdong Huang, Kevin Orton, Rachita Pandya, Jolinda Traugh

Translation is down regulated in response to a variety of stresses. Under moderate stress, p21-activated protein kinase (PAK2) is activated by Cdc42 and induces cytostasis, while caspase 3 cleaves and activates PAK2 under severe or prolonged stress conditions, leading to apoptosis. Our previous studies have identified that PAK2 inhibits translation through phosphorylation of initiation factor 4G (eIF4G) and a serine/threonine kinase Mnk1. Here, we performed systemic investigation of phosphorylation of ribosomal proteins by PAK2 using protein biochemical and cell biological techniques. It was found that three ribosomal proteins, S6 and S10 in the small ribosomal subunit and L34 in the large ribosomal subunit, were phosphorylated by PAK2. S10 and L34 were phosphorylated by both Cdc42-activated and caspase-cleaved PAK2, while S6 was phosphorylated only by caspase-cleaved PAK2. Unlike the S6 kinase, which phosphorylates five serine sites in S6, PAK2 phosphorylated only one site in S6, which was identified at serine 235 by mass spectrometry and Western blotting. This site was phosphorylated during early apoptosis following the treatment of 293T cells with H2O2, wherein PAK2 is activated by caspase 3. Addition of active PAK2 to rabbit reticulocyte lysate *in vitro* translation system resulted in the phosphorylation of ribosomal proteins S6 and S10, and the inhibition of protein synthesis. Reconstitution of the *in vitro* translation system with ribosomes phosphorylated by caspase-activated PAK2 *in vitro* inhibited translation by 33%. Our experiments also identified that active and inactive PAK2 were bound to intact ribosome and 40S ribosomal subunits, but not to 60S subunits, further explaining that the phenomena of PAK2 associated with ER *in vivo* could be mainly mediated by ribosomes. In conclusion, our study identifies a new mechanism that PAK2 can differentially phosphorylate different ribosomal proteins under different stress conditions, thereby leading to inhibiting translation to different extents or even regulating selective mRNA translation for various pathological responses.

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Abstract 1318

A Disordered Plant Microtubule Associated Protein Reorganizes Microtubules During Stress

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Alan Hicks, Wellington Leite, Shirish Chodankar, Jeremy Smith, Loukas Petridis, Hugh O'Neill

Companion of Cellulose Synthase 1 (CC1) has been recently identified to maintain cellulose synthesis during salt stress in Arabidopsis. CC1 is predicted to be a multi-domain protein with an N-terminal cytosolic disordered region (CC1NTD), a transmembrane region, and a C-terminal apoplastic region based on bioinformatics and structural modeling using Alphafold2. Previously, the disordered region of CC1 was found to interact with cortical microtubules in a similar fashion to intrinsically disordered human Tau protein however the structural basis for the interaction has not been investigated. In this work we investigated the solution structure of CC1NTD and its interaction with microtubules using small-angle X-ray and neutron scattering. Size exclusion chromatography – small-angle X-ray scattering analysis reveals that CC1NTD exists as a redox-dependent equilibrium mixture of monomers and dimers. SAXS shows that there is a structural rearrangement of microtubules in the presence of CC1NTD that supports bundling of microtubules. Using contrast variation SANS at the contrast match point of the microtubules, we observed the structure of deuterated CC1NTD and showed that the protein has a regular distribution across the microtubule lattice that is consistent with a tetragonal arrangement of CC1NTD around the microtubules. Overall, our study provides insights into how a disordered plant microtubule associated protein can affect the cortical microtubule network when plants are subjected to stress. Current research is now focused on understanding the structural remodeling of microtubules during this process.

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ASBMB

Abstract 1329

Isolation and Characterization of Protein Complexes of Overexpressed Human Serum Amyloid A from Cytokines-Induced Hepg2 Cells

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Elsie Parés-Matos

Serum Amyloid A (SAA) is an apolipoprotein found in the serum of many vertebrate species and is associated with the acute-phase reaction in the body with expression levels reaching up to a 1000-fold increase. The loss of its alpha-helix conformation during its expression peak is directly linked to secondary amyloidosis. Recent studies have been suggested to play a role in cholesterol and HDL metabolism, retinol transport and tumor pathogenesis. Moreover, high SAA concentration in blood have been correlated with severe symptoms or death in patients with COVID-19. However, how this protein is involved in so many diseases is uncertain or not completely understood. Therefore, the purpose of this research is to determine which protein-protein interactions with SAA occur in human cells, and to predict its biochemical role based on new discovered complexes. Two major isoforms overexpressed during an acute-phase reaction, human SAA1 and SAA2, are the focus of this study. Both are primarily produced in hepatocytes. HepG2 cells were cultured and induced with interleukin-1b, interleukin-6, LPS and retinol. Protein complexes associated with SAA will be isolated through a co-Immunoprecipitation technique, resolved by SDS-PAGE, and characterized by mass spectrometry. Our hypothesis focus on those protein complexes with SAA to explain how this protein lead other undiscovered metabolic pathways involved in both cellular and survival regulation.

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Abstract 1332

Histone Phosphorylation in ALS/FTD: New Opportunities in Neurodegenerative Disease

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Amyotrophic lateral sclerosis (ALS) is a fatal and incurable neurodegenerative disease that affects cells in the brain and the spinal cord. Frontotemporal dementia (FTD) involves progressive neuronal loss in the frontal and temporal lobes of the brain. ALS and FTD form a neurodegenerative continuum sharing pathological and genetic features. While most cases have no genetic basis, hexanucleotide repeat expansions (HREs) in C9orf72 are the most common genetic alteration in ALS/FTD. HREs lead to dipeptide repeat (DPR) proteins, which aggregate into neuronal inclusions. It is clear genetics alone does not explain the etiology of ALS/FTD. Is there a role for epigenetics in neurogenerative disease? Eukaryotic DNA is packaged into chromatin, a highly organized protein-DNA complex. Changes in the structure of chromatin are sufficient to cause heritable phenotypic changes termed epigenetic. Epigenetic mechanisms include the covalent post-translational modification of histone proteins. Exploiting a C9orf72 *S. cerevisiae* model overexpressing the DPR PR50 (Pro-Arg repeated 50x), we discovered a genome-wide 50% increase in the phosphorylation of Histone H3 on Serine 10 (H3S10ph). H3S10ph prevents the formation of transcriptionally-silent heterochromatin. Remarkably, we find corresponding increases in induced pluripotent stem cells (iPSC) and fibroblasts from C9 patients. Furthermore, both chemical inhibition and genetic manipulation of the yeast homolog of Aurora B, Ipl1, leads to a marked amelioration of DPR toxicity in yeast models. Altogether, our findings highlight a role for epigenetic mechanisms in ALS/FTD. Epigenetic processes are highly accessible targets for pharmaceutical treatments and thus they can lead to novel, alternative approaches in the treatment of ALS/FTD and other neurodegenerative diseases.

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Abstract 1333

A Structural Basis for Bifunctional and Simultaneous Inhibition of Neutrophil Serine Proteases by Members of a Staphylococcal Innate Immune Evasion Protein Family

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Timothy Herdendorf, Zheng-Qing Fu, Brian Geisbrecht

Extracellular adherence domain proteins (EAP) are a class of innate immune evasion proteins secreted by the human pathogen *Staphylococcus aureus*. EAP domains are potent and selective inhibitors of cathepsin-G (CG) and neutrophil elastase (NE), which are the two most abundant neutrophil serine proteases (NSPs). Previous work from our group has shown that the prototypical EAP domain protein, EapH1, relies on structural plasticity of a single inhibitory site to block activity of CG and NE. However, whether other EAP domain proteins follow similar structure/function relationships remains unclear. To address this question, we studied the inhibitory properties of the first (Eap1) and second (Eap2) domains of the modular Extracellular Adherence Protein of *Staphylococcus aureus* and determined their structures bound to CG and NE, respectively. Both Eap1 and Eap2 displayed high-affinity, time-dependent inhibition of CG and NE. However, whereas the structures of Eap1 and Eap2 bound to CG showed an overall inhibitory mode like that seen previously for EapH1, the structures of Eap1 and Eap2 bound to NE revealed a new inhibitory mode involving a distal region of the EAP domain. Interestingly, crystal structures of ternary complexes formed between CG/Eap1/NE and CG/Eap2/NE showed that each inhibitor could bind two NSPs concurrently. Subsequent enzyme assays confirmed simultaneous inhibition of each NSP without obvious loss of potency. Our work demonstrates that EAP domain proteins can form structurally divergent complexes with two target proteases that share high levels of sequence and structural homology to one another, and thereby inhibit activity of both proteases simultaneously.

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Abstract 1342

Effects of Antioxidant and Polyphenol-Rich Substances on Amyloid-Beta Plaque Aggregation in a *C. elegans* Model of Alzheimer's Disease

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Betsy Burgos, Shuhan Xie, Johanna Farkas

While Alzheimer's disease is one of the leading causes of death in the world, much is still unknown about the pathology of this devastating disease. Currently, the most widely accepted hypothesis is the amyloid cascade hypothesis, which states that amyloid beta plaque formation and tau protein tangles are likely the leading causes of neurodegeneration. But how exactly do these amyloid-beta plaques impede function, and how might we diminish the formation or even reduce existing plaques? Can these plaques in the nervous system be reduced in size? After these interventions, could neural function be regained as a result? Using genetically modified *C. elegans* with amyloid-beta promoting genes, we treated worms with natural polyphenol-and-antioxidant-rich substances–including pomegranate, raspberry, and cinnamon– to investigate how these substances affected muscular function, neuronal architecture, and amyloid-beta plaque buildup. We performed multiple assays using light microscopy, DiI staining, thioflavin S staining, and fluorescent microscopy to better understand the effects of these substances on the nervous system of *C. elegans*. Our results indicated that cinnamon has very strong potential neuroprotective benefits against amyloid-beta plaque aggregation. The experimental worms treated with cinnamon had significantly less plaques than experimental worms with no cinnamon. Additionally, we suspect that there may have been a reversal effect as well due to the fact that adult worms had less plaques than larval stage worms. This experiment supports other similar findings that name cinnamon with having strong correlations to neuroprotection. We hope our findings will promote more research to develop new potential drug therapies to slow down the overactivity of amyloid beta in neurodegenerative disorders.

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Abstract 1346

Structures of SegA and SegB proteins insights into chromosome segregation in archaea

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Daniela Barilla, Yuh-Ju Sun

Genome segregation is a vital process in all organisms. Chromosome partitioning remains obscure in Archaea, the third domain of life. Here, we investigated the SegAB system from *Sulfolobus solfataricus*. SegA is a ParA Walker-type ATPase and SegB is a site-specific DNA-binding protein. We determined the structures of both proteins and those of SegA-DNA and SegB-DNA complexes. The SegA structure revealed an atypical, novel non-sandwich dimer that binds DNA either in the presence or absence of ATP. The SegB structure disclosed a ribbon-helix-helix motif through which the protein binds DNA site-specifically. The association of multiple interacting SegB dimers with the DNA results in a higher-order chromatin-like structure. The unstructured SegB N-terminus plays an essential catalytic role in stimulating SegA ATPase activity and an architectural regulatory role in segrosome (SegA-SegB-DNA) formation. Electron microscopy results also provide a compact ring-like segrosome structure related to chromosome organization. These findings contribute a novel mechanistic perspective on archaeal chromosome segregation.

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Abstract 1369

The generation of an engineered HEK293T cell line bearing mutant NEMO incapable of binding to linear polyubiquitin

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Tom Huxford

NF-κB essential modulator (NEMO) is a necessary scaffold subunit of the Inhibitor of NF-κB Kinase (IKK) complex. IKK is central to the nuclear translocation of NF-κB, which is an inducible transcription factor that controls inflammatory gene expression. Activation of IKK catalytic activity in response to TNF-α and other canonical inducers of NF-κB requires formation of non-degradative linear polyubiquitin chains and their association with its NEMO subunit. Active IKK phosphorylates IκBα, an inhibitor protein of NF-κB, and triggers its degradation, thereby allowing NF-κB to enter the nucleus. Recent observations suggest that the IKK NEMO subunit, upon noncovalent association with linear polyubiquitin, mediates a second protein-protein interaction with the catalytic IKK2 subunit and "primes" the complex for activation. In order to investigate the direct involvement of NEMO in promoting catalytically active IKK, we have generated an engineered HEK293T cell line in which NEMO has been mutated to lack its ability to bind to linear polyubiquitin. We used the CRISPR prime editing system to mutate the HEK293T cell line and verified the introduction of the mutation through western blot and Sanger sequencing. In future endeavors, the cell line will be used to compare to the normal wild-type cell line and the same engineering approach will be used to generate different mutations to monitor NEMO-mediated signaling events.

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Abstract 1382

Visualization and functional annotation of coronavirus spike protein glycoshields

Shang-Te Danny Hsu, *Academia Sinica*

Tzu-Jing Yang, Pei-Yu Yu, Yu-Xi Tsai, Ning-En Chang, Kay-Hooi Khoo, Cyril Hanus, Mateusz Sikora

Spike proteins of coronaviruses are highly glycosylated and responsible for host recognition and viral entry. The glycans provide a camouflaging shield to help coronaviruses evade host immunity and, in some cases, modulate functional domain structures and dynamics pertinent to host recognition. However, the glycans are chemically and conformationally heterogeneous, making it challenging to determine the chemical compositions and conformations quantitatively. Combining cryo-electron microscopy, mass spectrometry, and molecular modeling, we systematically characterize a panel of spike protein variants of human and animal coronaviruses, including those of the variants of concern of SARS-CoV-2. We have established a robust workflow to quantify the heterogeneity of individual N-glycans by mass spectrometry. We also demonstrated the ability to visualize long glycan structures directly in regions where the dynamics are restricted. In places where the N-glycans are too dynamic, their structural information is generally lost after extended cryo-EM data processing that aims to achieve high resolution. To address this issue, we developed a computational tool called GlycoSHIELD to generate ensembles of glycan conformers to recapitulate the fuzzy structures that are in quantitative agreement with the experimental cryo-EM data. The ability to generate fully glycosylated spike protein models enables the prediction of hitherto unknown receptor and antibody binding sites.

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Abstract 1385

An evolutionary divergent thermodynamic brake in ZAP-70 fine-tunes the kinetic proofreading in T cells

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T cell signaling initiates by the formation of signalosome upon antigen binding to the T-cell receptor. The stability of the TCR–antigen complex and the delay between the recruitment and activation of each kinase determines the T cell response. These delays together establish a kinetic proofreading mechanism, regulating T-cell response. ZAP-70 is an essential non-receptor tyrosine kinase which initiates T-cell signaling. We observed the initial encounter complex formation between the ITAM-Y2P and tandem SH2 domain follows a fast-kinetic step, whereas the conformational transition to the holo-state follows a slow-kinetic step. But, how the delay initiated by ZAP-70 binding regulates the kinetic proofreading mechanism is poorly understood. Combining biochemical experiments and kinetic modeling, we identify a thermodynamic brake in the regulatory module of the tyrosine kinase ZAP-70, which determines the ligand selectivity, and may delay the ZAP-70 activation upon antigen binding to TCR. We show the initial encounter complex formation between the ITAM-Y2P and tandem SH2 domain follows a fast-kinetic step, whereas the conformational transition to the holo-state follows a slow-kinetic step. We further observed a thermodynamic penalty imposed during the second phosphate-binding event reduces the rate of structural transition to the holo-state. Phylogenetic analysis revealed the evolution of the thermodynamic brake coincides with the divergence of the adaptive immune system to the cell-mediated and humoral responses. In addition, the paralogous kinase Syk expressed in B cells does not possess such a functional thermodynamic brake, which may explain the higher basal activation and lack of ligand selectivity in Syk.

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Abstract 1386

Evolutionary Advantage to Protein Splicing in an Extreme Halophile

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Inteins are intervening sequences of protein that catalyze their own excision from and the ligation of the flanking sequences. Inteins may simply be molecular parasites, yet they have been preserved over the span of millions of years without being lost to evolution. Our goal is to understand whether there is an evolutionary advantage to protein splicing in *Halobacterium salinarum*. *H. salinarum* has two inteins: one that interrupts a cell division control protein and one that interrupts a polymerase II protein. We will create *H. salinarum* strains without each intein and compare the growth of the transgenic mutants to the wild type to assess the potential growth advantage posed by protein splicing via a pop-in/pop-out strategy. Because the inteins in *H. salinarum* do not contain a homing endonuclease domain, which could help to maintain the gene in the absence of positive selective pressure, we hypothesize that there will be no fitness cost to having the intein.

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Abstract 1390

Multiplex measurements of the impacts of ACE2 sequence and cell surface abundance during SARS-like coronavirus entry

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SARS-like coronaviruses, including SARS-CoV and SARS-CoV-2, encode spike proteins that bind human ACE2 protein on the cell surface to enter target cells and cause infection. The efficiency of virus entry depends on ACE2 sequence and expression levels in target cells. A small fraction of humans encodes variants of ACE2, thus altering the biochemical properties at the protein interaction interface. All humans possess cells with vastly differing amounts of ACE2 on the cell surface, ranging from cell types with high expression in the gut and lungs to lower expression in the liver and pancreas. Mastering our understanding of spike-ACE2 interaction and infection requires experiments precisely perturbing both variables. Thus, we developed a synthetic cell engineering approach compatible with high throughput assays for pseudotyped virus infection. Our assay system is capable of assessing both variables individually and in combination. We adapted an engineered HEK293T DNA recombinase landing pad cell line capable of expressing transgenic ACE2 sequences at highly precise levels. Infection with lentiviruses pseudotyped with the spikes of SARS-like coronaviruses revealed that high ACE2 abundance could mask the effects of impaired binding thereby making it challenging to know the role of affinity altering mutations during infection. We limited the ACE2 abundance on the cell surface by expressing transgenic ACE2 behind a suboptimal Kozak sequence,thereby altering its protein translation rate. This allowed us to understand how ACE2 sequence could impact its interaction with coronavirus spike proteins as two human ACE2 variants at the binding interface, K31D and D355N, exhibited reduced infection. Our experiments suggested that we need to better understand how ACE2 expression determines the susceptibility of cells for SARS-like coronavirus binding and infection. We thus created an ACE2 Kozak library consisting of ~4,096 Kozak variants, each conferring a different ACE2 protein translation rate thus resulting in a range of ACE2 steady-state abundances. Combining fluorescence-activated cell sorting and high-throughput DNA sequencing (FACS-seq) revealed the library to span two orders of magnitude of ACE2 abundance. Challenging this library of cells with spike pseudotyped lentiviruses revealed how ACE2 abundance correlated with infection rate. The library-based experiments yielded a dynamic range wider than traditional single sample infection assay, likely more representative of infection dynamics *in vivo*. Now that we have characterized the impacts of ACE2 abundance on infectivity in engineered cells, our next goal is to expand the comparison to physiologically relevant cells with endogenously expressed proteins. Modulating protein

abundance levels will be key to creating maximally informative functional assays for any protein in cell-based assays, and we have laid the groundwork for being able to simultaneously test the impacts of protein abundance and sequence in combination for proteins involved in diverse cellular processes.

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Abstract 1393

The Sequence Basis for Selectivity of Ephrin B2 for its Receptors and Henipaviral G Proteins

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Design of selective ligands for protein-protein interactions can be achieved through deep mutational scanning. Ephrin B2 is a ligand for six Eph receptors in humans as well as functions as the cell entry receptor of Nipah virus (NiV), a highly pathogenic zoonotic virus from the Henipavirus genus which causes deadly diseases in humans and has epidemic potential. To understand the sequence basis of promiscuity for EFNB2 binding to the attachment glycoprotein of NiV (NiV-G) and Eph receptors, we performed deep mutagenesis of the receptor binding domain of EFNB2 to identify mutations that enhance binding to NiV-G over EphB2, one of the six Eph receptors. The mutations highlight how different EFNB2 conformations are selected by NiV-G versus EphB2. One mutant of interest possessed pan-specificity to the attachment glycoproteins of closely related Henipaviruses and had no or very weak binding to four Eph receptors. However, this EFNB2 variant had high residual binding to two Eph receptors, EphB3 and EphB4, which may limit its safety if utilized as a soluble decoy receptor for therapy and prophylaxis. A second deep mutagenesis of EFNB2 was performed with a fixed single substitution to identify potential combinatorial mutations to further enhance specificity to NiV-G over EphB3 and EphB4. A triple mutant of soluble EFNB2 exhibited substantially diminished binding to Eph receptors but maintained binding, albeit reduced, to NiV-G. This protein mimic of EFNB2 could be potentially used for virus neutralization. In conclusion, we demonstrate how substitutions at the shared binding interface of a promiscuous ligand can influence conformational selectivity and specificity for multiple receptors.

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Abstract 1395

ATP-competitive and allosteric inhibitors induce differential conformational changes at the autoinhibitory interface of Akt1

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The protein kinase Akt is a master regulator of pro-growth signaling in the cell. Akt is composed of a kinase domain and a pleckstrin homology (PH) domain and is activated through its targeted recruitment to the plasma membrane by phosphoinositides which lead to the disruption of the autoinhibitory interface between the kinase and PH domains. This then leads to activating phosphorylation of the activation loop and hydrophobic motif. Hyper activation of Akt is common in oncogenic transformation, with multiple oncogenic activating mutants identified in Akt. This has driven the development of potent and selective ATP-competitive and allosteric inhibitors for Akt as therapeutics, with them in advanced stages of clinical trials. Paradoxically, some ATP-competitive Akt inhibitors cause hyperphosphorylation of Akt. Here, using hydrogen deuterium exchange mass spectrometry (HDX-MS), we interrogated the conformational changes induced upon binding to Akt ATP-competitive inhibitors (A-443654, Uprosertib, and Capivasertib) and the Akt allosteric inhibitor MK-2206. We compared the conformational changes that occurred for ATP-competitive and allosteric inhibitors in three different states of Akt: i-inactive monophosphorylated, ii-partially active trisphosphorylated [T308, T450, S473], and iii-fully activated, trisphosphorylated bound to phosphoinositide (PIP3) containing membranes. The allosteric MK-2206 inhibitor results in large scale allosteric conformational changes in all states and restricts membrane binding through sequestration of the PH domain. Binding of the ATP competitive inhibitors led to large scale allosteric conformational changes in both the monophosphorylated and phosphorylated states, leading to an alteration in the autoinhibitory PH-kinase interface. We also observed increased protection in the PH domain upon membrane binding in the presence of A-443654, suggesting that the PH domain is more accessible for membrane binding. This work provides unique insight into the autoinhibitory conformation of the PH and kinase domain and dynamic conformational changes induced by Akt inhibitors and has important implications for the design of Akt targeted therapeutics. Work from "ATP-competitive and allosteric inhibitors induce differential conformational changes at the autoinhibitory interface of Akt" by Shaw et al. (2022), bioRxiv https://doi.org/10.1101/2022.07.14.499806

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Abstract 1408

A Novel Regulation of p53 Protein Functions through Citrullinaiton by Peptidylarginine Deiminase 4

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Chien-Yun Lee, Guang-Yaw Liu, Hui-Chih Hung

p53 is a widely studied transcriptional factor and is also a well-known tumor suppressor, the regulation of p53 is quite complicated. In the previous studies, it has been reported that p53-targeted gene expression can be downregulated by peptidylarginine deiminase 4 (PAD4) through the interaction of p53, PAD4, and histone deacetylases (HDACs). PAD4-catalyzed citrullination has been involved in various cellular processes, such as inflammation, apoptosis, and epigenetic regulation of histone-related gene expression, PAD4 also has been found dysregulation and overexpression in many cancers, indicating that PAD4 is highly associated with tumorigenesis. Although PAD4 can decrease the p53 downstream gene expression through interacting with p53, the direct evidence of p53 and PAD4 interaction was rare, hence, we further discussed the relationship between p53 and PAD4. In this study, we revealed that p53 can be citrullinated by PAD4, and eight citrullination sites were identified by LC-MS/MS, most of them are located at the core DNA-binding domain of p53, and citrullination will lead to dysfunctions of p53 including loss of DNA-binding ability and impairment of its quaternary structure formation. In contrast, inhibition of PAD4 can upregulate p53-targeted gene expression, and further induce cell growth arrest, cell apoptosis, and promote cellular DNA repair. These findings provide another mechanism of how PAD4 regulates p53-targeted gene expression by citrullinating p53, which enhances the link between PAD4 and tumorigenesis.

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Abstract 1411

The Possible Effects of Rieske Reduction Potential on Superoxide Formation in Complex III

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The electron transport chain utilizes four protein complexes to generate an electrochemical gradient necessary for ATP synthesis. Complex III contains the Rieske protein that transports one electron via an [2Fe-2S] cluster from quinol to cytochrome c1. Through what is known as the quinol cycle, the successful transfer of electrons relies on matched reduction potentials between quinol, Rieske, and cytochrome c1 which drives the forward electron passage. If the reduction potential of Rieske is too low, it may inefficiently accept the electron from quinol, allowing the electron to react with molecular oxygen and produce superoxide. Superoxide is a reactive oxygen species (ROS). Thus, a mismatch in reduction potential between the Rieske protein and quinol may serve as a possible source of endogenous superoxide. To investigate this hypothesis, a two-pronged approach is being employed. The first part focuses on measuring the reduction potential of the isolated *Saccharomyces cerevisiae* Rieske protein (ScRieske) *in vitro* via cyclic voltammetry. Several isolated ScRieske mutants have been produced, which alter different amino acids predicted to change the reduction potential of Rieske. The second part of the project centers on measuring the levels of superoxide produced by intact *S. cerevisiae* Complex III (ScComplex III) *in vivo* using fluorometric confocal microscopy. In this part, the same mutants from the first part are being produced in ScComplex III, and are being analyzed using growth assays to determine cell health and confocal microscopy to quantify superoxide formation.

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Abstract 1422

Mutating D111 in the CuA protein to Determine the Reactivity of Ligating Histidines to Chemical Modifiers

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The electron transport chain (ETC) is a series of four protein complexes that couple redox reactions and the formation of an electrochemical gradient that leads to the formation of ATP. The electrons go through a chain of proteins (Complexes I- IV) with increasing reduction potentials and causes a release in energy. Complex IV, oxidizes cytochrome c and transfers the electrons to O2 , the final electron acceptor. Complex IV in *Thermus thermophilus* contains two heme groups and two copper sites in Subunit I and Subunit II. The primary focus for this project is Subunit II, where the soluble domain of the protein contains the binuclear CuA site. The CuA center contains two copper ions bridged by two cysteine thiolates; each copper is additionally coordinated by two histidine nitrogen atoms. Experiments have focused on the copper site and reactivity of the ligating, solvent exposed His 157. Asp 111 (D111) is hypothesized to have a role in the reactivity of the ligating, histidine 157 because D111 is hydrogen bonded to the other ligating histidine. By mutating Asp 111 to Asn, Ala, and Val, the change in proton exchange and reactivity of the histidine 157 can be determined by chemically modifying the mutants with diethyl pyrocarbonate (DEPC), 4-hydroxyl-trans-2-nonenal (HNE), and 4-oxo-2-nonenal (ONE). DEPC is a commonly used chemical modifier but is not present in biological systems. HNE and ONE are endogenous lipid peroxidation products. High levels of reactive oxygen species oxidize nearby membrane lipids. HNE and ONE are such products that act as toxic second messengers of oxidative stress and covalently modify lipids, proteins, and DNA. The modifications on TtCuA mutants by DEPC, HNE and ONE will provide more conclusions about the reactivity of the ligating histidines and thus possible sites of modification *in vivo* during oxidative stress.

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Abstract 1429

The interplay of casein kinase II and ankyrin G with the key renal ion channel, ENaC, in controlling blood pressure

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An estimated 1.28 billion people worldwide have hypertension, according to a 2021 WHO report. Hypertension is a major contributor to heart disease, and thus associated with premature death. A major goal of the WHO is to reduce the prevalence of hypertension by 33% between 2010 and 2030. The proper control of blood pressure partly depends on Na+ homeostasis. In the distal nephrons, Epithelial Na+ Channels (ENaC) play a role in balancing Na+ excretion with Na+ reabsorption. Large increases of Na+ reabsorption can increase blood pressure. Understanding the fine details of signaling pathways acting on ENaC can help improve therapies to help achieve the goal of reducing the prevalence of hypertension. ENaC is a heterotrimeric channel comprised of alpha, beta, and gamma subunits. We showed that casein kinase II phosphorylates Serine 631 (S631) of the intracellular C-terminus of the beta subunit of ENaC (betaC). Phosphorylation of S631 appears to be necessary for ankyrin G (AnkG) to stabilize ENaC at the membrane. S631 lies within a domain of the ENaC betaC that resembles an "SD-ESD" motif on the sodium channel Nav1.2 shown to also bind AnkG. We hypothesize that AnkG directly binds this similar "SD-ESD" site on ENaC betaC. We use structural and biochemical methods to show the precise binding domains between AnkG and ENaC that ultimately lead to the stabilization of ENaC at the luminal side of kidney tubules. The results of this study could help lay the foundation for developing therapeutics to target these ENaC-AnkG interactions to treat and prevent hypertension and heart disease.

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Abstract 1433

Characterizing the interaction of GATA-4 with the Myosin Chaperone, Striated Muscle UNC-45

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Andres Oberhauser

Striated muscle UNC-45 (SM UNC-45 or UNC-45B) protein acts as a chaperone for cardiac and skeletal muscle myosins, regulating their folding, assembly into thick filaments, interaction with other sarcomeric proteins, and degradation. GATA-4 is a transcription factor that regulates the expression of several cardiac muscle genes, including myosin. A previous study by Chen et al (2012, doi:10.1242/jcs.106435) demonstrated for the first time that SM UNC-45 (UNC-45B) physically interacted with GATA-4 and enhanced its transcriptional activity in an overexpression experiment. The current study aims to (1) identify the specific domain in UNC-45B that interacts with GATA-4, and (2) characterize the nature of this interaction. Protein-protein docking coupled with pull-down assays using native and mutant proteins identified the UCS domain of UNC-45B as the preferred binding region of GATA-4. Ongoing experiments include surface plasmon resonance spectroscopy, atomic force microscopy (AFM) and chaperoning assay to characterize the nature of the interaction and obtain binding affinities. By modulating GATA-4 transcriptional activity, UNC-45B shows potential to exert both short-term (protein level) and long-term (gene level) controls over myosin and therefore muscle structure and function.

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Abstract 1435

Production of Lymphocyte Antigen 6 Proteins – Targets for Cancer Treatment

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The lymphocyte antigen 6 gene (LY6) family of proteins are involved in cell proliferation, macrophage activation, and immune cell maturation. Their location on chromosome 8q24 is frequently amplified in human cancer, and the increased expression of these proteins is associated with poor outcomes in ovarian, colorectal, breast, bladder, and pancreatic cancer. Ovarian cancer often goes undetected which leads to its high mortality, while colorectal and pancreatic cancer are the second and third leading causes of cancer death in the United States. The increased expression in fatal cancers makes these proteins good candidates of new anticancer drugs. In our research, we focused on two members of this family: LY6D and LY6K. The high expression of LY6D is correlated with poor clinical outcomes in pancreatic cancer, and LY6K is required for *in vivo* tumor growth in breast cancer. We aim to determine the structure of these proteins to understand their role in invasive cancer and to develop anticancer drugs that target these proteins using recombinant protein expression and purification techniques. We developed and optimized procedures to obtain soluble recombinant LY6D and LY6K in *E. coli*. *In vitro* studies have been performed to characterize the biochemical properties of these polypeptides, specifically oligomeric states. Furthermore, two different approaches are simultaneously being carried out to determine the tertiary structure of these proteins: screening for protein crystals to perform X-ray crystallography experiments and production of the 15N labeled protein to perform NMR experiments. NMR experiments indicate the presence of partially folded LY6D; however, aggregation of LY6K poses a challenge to structural studies. Complementary to these studies, in silico experiments are being used to identify small molecules with anticancer properties that bind with LY6D. Computational studies indicate a potential binding site in LY6D. In the case of LY6K, computational studies are focused on understanding the interactions with a small molecule shown to have anti-cancer properties *in vivo*, and to generate fluorophore and biotinylated derivatives of this drug for further optimization. These studies aim to elucidate the LY6D and LY6K interactions with the drug in order to suggest innovations in cancer therapy.

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Abstract 1445

A New Player in Bacterial Zinc Import, Study of Structure and Function of the Unusual Solute Binding Protein ZrgA from *Vibrio cholerae*

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The human host employs several mechanisms to withhold the essential metal zinc from pathogenic bacteria to reduce virulence during infection. Bacterial ATP binding cassette (ABC) transporters are critical to overcome this limitation as evidenced by dramatic decreases in virulence for knockout mutants in many human pathogens. These transporter systems rely on an extracellular solute binding protein (SBP) to bind zinc with high affinity and specificity. The causative agent of cholera, *Vibrio cholerae* has two ABC transporters for zinc, the well-characterized znuABC and a more recently identified zrgABCDE. The zrgA gene encodes a periplasmic protein conserved in hundreds of organisms near other ABC transporter genes, suggesting a function as an SBP to these systems. However, it bears no sequence similarity to known SBPs, and its independent function has not been evaluated. The ZrgA homologue from *Pseudomonas aeruginosa* was recently shown to bind zinc but shows no structural similarity to known SBPs. Thus, ZrgA may represent a new family of zinc SBPs conserved in a number of human pathogens that require further study. Here we show that the zrgA gene is critical to the zinc import function of zrgABCDE operon in *V. cholerae*, verifying its likely function as a zinc SBP through *in vivo* experiments. We also observe high-affinity zinc binding *in vitro* through the use of competitive binding assays, further defining its role as an SBP. ZrgA proteins possess a low-complexity region typically rich in His residues whose function has not been previously evaluated. In *V. cholerae*, this region is approximately 60 residues in length. We show that it is not essential to the function of ZrgA, but it does provide additional low-affinity zinc binding sites *in vitro*. Finally, the crystal structure of a ZrgA mutant in both apo and holo forms was solved through X-ray crystallography. The observation of multiple zinc binding sites, several of which are not conserved in other ZrgA homologues, was consistent with solution data and reveals diversity within the ZrgA family. This project further characterizes a new family of zinc solute binding proteins through an understanding of its structure, how that impacts coordination of zinc and its ability to act as a transporter *in vivo*.

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ASBMB

Abstract 1446

A proteomic approach to determining whether temperature-sensitive yeast Hsp90 alleles that exhibit similar growth defects have differing effects on client proteins

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The abundant, essential cytosolic Hsp90 facilitates the folding and activation of hundreds of client proteins in an ATP-dependent folding pathway facilitated by distinct cochaperones that bind Hsp90 as it cycles through 'open' and 'closed' conformations. We previously identified a series of yeast Hsp90 mutants that appear to disrupt either the 'loading', 'closing' or 'reopening' events, and showed that the mutants had differing effects on activity of some clients. Effects of over-expression or deletion of genes encoding the Sti1, Cpr6, Sba1, Aha1 and Hch1 cochaperones also correlates with the conformational cycle, with a phenotypic shift coinciding with formation of the closed conformation. We conducted a quantitative proteomic analysis of yeast strains comparing extracts of yeast expressing wild-type Hsp90 or nine different mutant alleles that result in similar growth defects. Out of 2482 proteins in our sample set (approximately 38% of yeast proteins), we observed statistically significant changes in abundance of 350 (14%) of those proteins ((>log2fold > = 1.5, P). Many of the 350 proteins have previously been identified as Hsp90 interactors. Despite resulting in overall similar growth defects, each mutant had a different profile of affected proteins. For example, one mutant affected abundance of 244 proteins, while another affected only 13. Analysis of the differential effects of the Hsp90 mutants will test the hypothesis that Hsp90 mutants that affect similar stages in the folding pathway have similar effects on client activity and may provide new insights into how to achieve selective inhibition of Hsp90 functions.

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Abstract 1449

Probing Local Protein Solvation States in Adenylate Kinase with 4-Cyano-L-Phenylalanine

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Non-canonical amino acids (ncAAs) containing vibrational reporters such as nitrile and azide groups have proven to be effective, site-specific reporters of local protein environments. In particular, the vibrational reporter ncAA 4-cyano-L-phenyl-alanine (pCNPhe) is advantageous due to the sensitivity, position, and extinction coefficient of the nitrile symmetric stretching frequency in addition to not typically suffering from accidental Fermi resonance as compared to 4-azido-L-phenyl-alanine. Here, pCNPhe was site-specifically incorporated into Adenylate Kinase (AK) individually at a number of sites predicted to have a range of local protein environments using the Amber codon suppression methodology. These protein constructs were then interrogated with temperature-dependent IR spectroscopy to measure the temperature dependence of the nitrile symmetric stretching frequency of pCNPhe at each site of the protein in the open conformation of the protein. These temperature dependencies in addition to the room temperature nitrile stretching frequencies were then used to determine the local solvation environment of the nitrile group in each of these constructs. These results in addition to progress towards determining the X-ray crystal structures of these protein constructs will be presented.

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Abstract 1450

Tuning the Gas Binding Affinity of Cs H-NOX Using Tyrosine Analogs

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Caldanaerobacter subterraneus heme-nitric oxide and/or oxygen binding domain (Cs H-NOX) is a thermophilic heme protein that binds to small gas molecules including oxygen, nitric oxide, and carbon monoxide. Here, non-canonical amino acids (ncAAs) were utilized to either probe the local solvation environments in the protein or to modify the functionality of the protein. Specifically, the ncAA vibrational reporter 4-cyano-L-phenylalanine (pCNPhe) was site-specifically incorporated into the protein at a number sites using the Amber codon suppression methodology, including surface and buried sites in the protein. Temperature-dependent IR spectroscopy was then utilized to measure the temperature dependence of the nitrile symmetric stretching frequency of pCNPhe which was correlated to local solvation environment in conjunction with X-ray crystal structures of the protein constructs generated. Additionally, tyrosine analogs were individually incorporated at site 140 which is a tyrosine in the native protein structure. Y140 is involved in a hydrogen bonding interaction with oxygen bound to the heme iron. Thus modulation of the pKa of the phenolic hydrogen is predicted to impact the strength of this interaction and thus the affinity of the protein to oxygen binding. Results from probing local protein environments with ncAAs and modulating oxygen affinity will be presented.

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Abstract 1452

Modeling the binding of ω-conotoxin and other toxins to the N-type voltage-gated calcium channel

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Approximately 1.5 billion people in the world suffer from chronic pain, persistent pain that carries on for longer than 12 weeks despite medication or treatment. Management of chronic pain typically includes the use of non-steroidal anti-inflammatory drugs (NSAIDs) or prescription pain medications, including opioids. An alternative therapy derived from conotoxins, toxins released from marine predatory snails in the family Conidae, was approved for the treatment of severe chronic pain in 2004. This pharmaceutical has the trade name Prialt and is also known as ziconotide. Once ziconotide is in the human body, it acts as a channel blocker of the N-type voltage-gated calcium channels, also known as Cav2.2. The literature and related PDB files were manipulated using PyMol and Jmol to create a 3D-printed model to explain the molecular story behind how a particular conotoxin binds to a calcium-gated ion channel. Additionally, computer visualization tools were used to show how several related toxins from other organisms would be expected to dock to the calcium ion channel. The 3D-printed model highlights specific features that contribute to the ω-conotoxin (MVIIA) binding to the calcium channel alpha 1B subunit as described in the literature (PDB: 7MIX). These conotoxins have a very characteristic disulfide bond linkage pattern which plays a role in the correct folding of the peptide and stabilization of its structure. In MVIIA, the non-cysteine amino acids form unstructured loops affecting binding affinity and calcium channel-blocking activity. Of particular interest is the second loop located between Cys8 and Cys-15. It appears to be exceptionally important in directing selectivity toward N-type calcium channels and away from P/Q-type calcium channels. Ziconotide does not directly seal the entrance to the vestibule of the selectivity filter, but it blocks ion entrance by neutralizing the outer electronegativity and sterically hindering the ion access path to the entrance of the selectivity filter. Salt bridges are formed between Arg10 and Tyr13 on ziconotide and Asp664 of the channel. Four of the eight ziconotide-coordinating residues, Thr643, Asp1345, Lys1372, and Asp1629 in Cav2.2 are not conserved in other calcium channels which may explain the subtype specificity of pore blockage by ziconotide. The EEEE motif consisting of Glu314, Glu663, Glu1365, and Glu1655, determines the Ca2+ selectivity. Also included in the model are the receptor's alpha helices and bound calcium ion. The N terminus and C terminus of the receptor are labeled in blue and red respectively to orient the model. No crystal structures are available for ω-conotoxins bound to several other types of N-type calcium channels. To investigate the potential calcium channel blocking properties of conotoxins MVIIC,

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GVIA, MoVIB (from the cone snail, Conus magus) and ω-agatoxin IVA (from the spider, Agelenopsis aperta), the computer-based tool ROSIE was used to simulate binding of these peptides to the Cav2.2 channel. As expected, the toxin shown in the crystal structure of 7MIX, bound best to the Cav2.2 channel. The toxins MVIIC, GVIA, and MoVIB bound with lower affinity. The agatoxin IVA did not have any relevant binding to this calcium channel. Overall, protein modeling allowed for a deeper understanding of how conotoxins bind to and block the calcium channel possibly leading to additional therapeutic approaches to pain relief.

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Abstract 1470
Probing the Pal-TolB Interaction in *E. coli*

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Peptidoglycan Associated Lipoprotein (Pal) is a protein found in the periplasmic domain of Gram-Negative bacteria, such as *Escherichia coli* (*E. coli*). In *E. coli*, Pal acts as a structural protein as it is tethered to the outer membrane (OM) through its lipid moiety and non-covalently binds to peptidoglycan (PG). Pal also associates with other OM and periplasmic proteins as part of the Pal-Tol complex, which is known to provide the bacterial cell with structural integrity and to play a crucial role in cell division by assisting in the constriction of the OM. For these reasons, we see Pal as an antibiotic target. To better understand the Pal-TolB interaction, which is known to be important for constriction, we created two site-directed mutants of Pal (K150E and G113P) with the goal of disrupting the Pal-TolB interaction. Recombinant wild-type and mutant proteins were expressed in *E. coli*. The effects of the mutations on the Pal-TolB interaction were determined using enzyme-linked immunosorbent assays (ELISA). Preliminary results of this study suggest that K150 and G113 dictate at least some of the Pal-TolB interaction. Future work will determine how mutations that decrease the Pal-TolB interaction affects cell division in *E. coli* cells.

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Abstract 1473

Modeling Binding of Beta-site Amyloid Precursor Protein Cleaving Enzyme 1 (BACE1) Inhibitor Aminoquinoline (68 K) for Possible Treatment of Alzheimer's Disease

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Alzheimer's Disease (AD), affecting approximately 24 million people worldwide, is characterized by the formation of amyloid-β plaques within the brain. Alzheimer's research has been focused on limiting amyloid-β production through developing inhibitors for the enzymes needed within the amyloid cascade. This project focuses on the aminoquinoline class of inhibitors, of which 68 K (PDB: 5i3Y) is the most effective because of its strong Kd and IC50 values. The students of the Honors Protein Modeling class at Nova Southeastern University modeled the interaction between Beta-site Amyloid Precursor Protein Cleaving Enzyme 1 (BACE-1) and 68 K. Using Jmol a model was developed, and 3D printed to show how the inhibitor (68 K) fit into the enzyme's active site. This model highlights important aspects of the interactions between the ligand and the BACE-1 enzyme. 68 K has strong interactions with 32 amino acid residues in BACE1, some of which are intertwined with one another. For example, BACE-1's residues Val69, Pro70, and Tyr71 are known collectively as "the flap". "The flap" is a β-hairpin loop structure that is positioned directly over BACE-1's catalytic dyad, a group of amino acids within the active site of the enzyme. "The flap" is also responsible for regulating access to the enzyme's catalytic dyad (Asp 32 and Asp 228) by a given substrate (or inhibitor). Researchers found the inhibitor 68 K to have interactions with the flap which maximizes the strength of the interaction with BACE-1 residues, thus minimizing the distance between the inhibitor's various functional groups and accommodating their specific polarities. Being able to visualize the protein structure using a 3D model aids in the understanding of how the ligand inhibits this enzyme leading to the progression of AD.

This work was made possible by funding through the National Science Foundation, Division of Undergraduate Education (NSF-DUE) grant number 1725940 for the CREST Project. Nova Southeastern University's Farquhar Honors College and Dept. of Biological Sciences also provided support. Protein model printing was made possible by 3d Molecular Designs.

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Abstract 1474

Regulation of Cullin 2-RING ubiquitin ligases by NEDD8 and CAND1

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Through recruiting interchangeable substrate receptor modules (SRMs) to the cullin (CUL) core, cullin-RING ligases (CRLs) catalyze the ubiquitination and degradation of diverse proteins that play key roles in a myriad of biological processes in human cells. As the founding member of CRLs, the CUL1-based CRL1s are known to be activated by both CAND1, which exchanges the SRMs associated with the common CUL1 core, and NEDD8 conjugation (neddylation), which modifies CUL1 and alters CRL1 conformation to promote substate ubiquitination. In comparison with CRL1s, CRL2s comprise the CUL2 core that is homologous to CUL1, and SRMs whose composition differ from CRL1s. Because of the structural difference, it is unclear if NEDD8 and CAND1 regulate CRL1 and CRL2 in similar fashions. To uncover mechanisms regulating CRL2s, we studied the CRL2VHL, a ubiquitin ligase well known for targeting HIF1α for ubiquitination and degradation, and for PROteolysis TArgeting Chimeras (PROTACs) induced degradation of disease-causing proteins. We found that neddylation promoted the *in vitro* ubiquitination of the full-length HIF1α and the degron peptide of HIF1α to similar extents, demonstrating that neddylation activates CRL2VHL-dependent ubiquitination regardless of the substrate sizes. In HEK293 human cell-based cycloheximide chase assays, eliminating neddylation failed to stabilize HIF1α due to VHL-independent degradation, but it stabilized the truncated HIF1α containing only the C-terminal Oxygen-Dependent Degradation (C-ODD) domain. This unexpected finding suggests that the cellular activity of CRL2VHL is better reflected by the degradation of C-ODD than that of HIF1α, and we thus analyzed the degradation of C-ODD in HEK293 cells with or without CAND1-knockout (KO). Surprisingly, C-ODD was degraded faster in the KO cells, and similarly, the PROTAC-induced degradation of CRL2VHL neosubstrates also became faster in the KO cells. This inhibitory effect of CAND1 on CRL2-dependent degradation was then explained by our kinetic measurements of CRL2VHL and CAND1 interactions using *in vitro* Förster resonance energy transfer (FRET) assays. We found that CAND1 dramatically increased the dissociation rate of the CRL2VHL complex but hardly accelerated the assembly of stable CRL2VHL, leading to an overall reduction in CRL2VHL assembly. We then hypothesized that this inhibitory effect is important for CRL2 activity in human cells because it allows only proteins that bind CRL2 tightly enough to be ubiquitinated, and thereby, it enhances CRL2 substrate specificity. Using PROTACs that recruit neosubstrates to CRL2VHL with different affinities, we found evidence supporting this hypothesis, showing that eliminating CAND1 from HEK293 cells shortened the half-life of lower affinity substrates but had no effect on the stability of higher affinity substrates. Taken together, we conclude that instead of being an exchange factor that activates other CRLs, CAND1

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inhibits the dynamic assembly of CRL2 to prevent the ubiquitination of proteins that weakly bind to CRL2, presenting a mechanism that contributes to the specificity of the CRL2 system and limits the efficacy of CRL2-based PROTACs.

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Abstract 1485

Structure guided mutations of the ubiquitin conjugating enzyme UbcH5b

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Ubiquitination is a crucial process in maintaining cellular homeostasis, controlling cell signaling and cellular environment. The proteins E1 - ubiquitin-activating enzyme, E2 - ubiquitin-conjugating enzyme; (UbcH5b in this study), and E3 - ubiquitin ligase each play major roles in the process to transfer a ubiquitin to a targeted substrate protein. The C-terminus of Hsp70 interacting protein (CHIP) acts as an E3 ubiquitin ligase which coopts molecular chaperone functions to direct the degradation of misfolded or unstable proteins. The C-terminal U-box domain of CHIP recruits the E2 enzyme in the form of the E2∼Ubiquitin conjugate. Although structures exist for CHIP in complex with the E2 enzyme UbcH5b, no structures for CHIP in complex with an E2∼ubiquitin conjugate are available. A structure of the complex would greatly aid understanding how CHIP facilitates the ubiquitination of targeted substrates. To facilitates structural studies, we have designed structure guided mutants of UbcH5b which strengthen the affinity for CHIP, in an effort to promote formation of the CHIP/UbcH5b∼ubiquitin complex. Results from these efforts include analysis of patterns of polyubiquitination, UbcH5b∼ubiquitin conjugate formation, and variations in affinity between partners within the larger CHIP/UbcH5b∼ubiquitin complex.

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Abstract 1497

The structural variance of amyloid peptides influences oxidative activity in the human brain

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The brain of an Alzheimer's Disease (AD) patient is characterized by the formation of abnormal levels of amyloid-beta (Aβ) plaques that cause oxidative stress. However, the mechanism of the Aβ peptide, and therefore the exact pathogenesis of AD, is yet to be fully understood. This study investigates how the presence, frequency, and position of amino acids like Histidine (His) within the soluble Aβ peptide sequence variants play a critical role in influencing their metal binding and redox properties. Performing Cu(II) titrations on these variants and examining their UV spectra confirmed the strong metal-binding capabilities of His, as evidenced by their metal-binding geometries influenced by the position of His within the sequences that were directly correlated with their molar absorptivity at a specific wavelength. A higher molar absorptivity indicated a similar binding environment for copper in the peptide sequences compared to Aβ. The oxidative cleavage of Cu(II)-peptide complexes that exhibited greater metal-binding capabilities was examined through gel electrophoresis using a commercially available plasmid, pUC18, in the presence of hydrogen peroxide at similar conditions used in the oxidative DNA cleavage studies for the CuAβ1-20 and CuAβ1-16 soluble fragments (da Silva, Tay, and Ming, 2005) to compare their DNA cleaving patterns. Their oxidative activity was also dependent on the positioning of His: peptide sequences flanked by His in certain positions could form active coordination complexes, whereas peptides with His in a different location displayed little to no oxidative activity, therefore indicating the inability to form substrate binding sites. The fragments RHHPPHHEF-NH2, AC-HHFDEDGDGTHH-NH2, and AC-RHHPPHHEF-NH2 displayed the greatest levels of oxidative activity, and further DNA gel analysis will be performed in experimental environments mimicking the brain to better understand the activity of Aβ in AD processes. Through identifying the Aβ sequence variants most active in contributing to oxidative activity, critical insight can be developed regarding AD treatment and potential contributions in fields beyond neurodegenerative diseases.

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Abstract 1518

BNIP3 protein abundance is controlled by phosphorylation

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Mitochondria found in almost all eukaryotic cell types, house numerous metabolic and signaling processes. Mitochondria are thus tightly regulated by cells facing stresses, such as changing nutrient environments and hypoxia. During hypoxia, cells undergo a metabolic shift to glycolysis, and excessive mitochondrial mass is degraded through mitophagy to provide nutrients and other resources to the cell and to limit the extent of reactive oxygen species production. BNIP3 (BCL2/Adenovirus E1B 19 kDa protein interacting protein 3) is a mitophagy receptor transcriptionally upregulated during hypoxia and is localized to the outer mitochondrial membrane to facilitate LC3 recruitment. While the transcriptional regulation of BNIP3 is well understood, the current understanding of BNIP3 abundance regulation at the protein level is incomplete. Thus, the objective of this study is to understand the the molecular mechanisms underlying the regulation of BNIP3 protein accumulation and turnover. Here, using immunoblotting and transcript analysis, we find that BNIP3 protein abundance is influenced by the mitochondrial phosphatase PPTC7 (protein phosphatase targeting CoQ7) post-transcriptionally. Phosphoproteomics datasets generated in Pptc7 knockout (KO) mouse systems show that Bnip3 is hyperphosphorylated in these systems. Our data suggest that at least a subset of these phosphorylation sites influence BNIP3 protein stability, likely through mediating its degradation through the ubiquitin proteasome system. Our data reveal a novel regulatory network that influences BNIP3 protein stability post-transcriptionally, independent of hypoxic insults. These data reveal novel post-translational mechanisms by which cells alter the abundance of stress-response proteins to regulate the mitochondrial population.

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Abstract 1532

Exploring structural differences between antagonistic peptides for the development of orally bioavailable PCSK9 inhibitors

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Familial hypercholesterolemia (FH) is an autosomal genetic disease that causes elevated blood levels of low-density lipoprotein (LDL). One of the leading causes of FH is gain-of-function mutations in the gene coding for proprotein convertase subtilisin/kexin type 9 (PCSK9). The PCSK9 protein binds to LDL receptors (LDLR) on the surface of hepatocytes and promotes their degradation, preventing the recycling of LDLRs and thus increasing LDL blood levels. Monoclonal antibody therapies that bind to PCSK9 inhibiting LDLR binding are currently only available as an injection. However, several orally bioavailable PCSK9 inhibitors have been formulated and are undergoing clinical trials. One such therapy contains small-molecule-peptide inhibitors that bind to a cryptic site (N-terminal groove) adjacent to the LDLR binding site located in the catalytic domain. A helical region (S153-I161) is contained within this groove with conformational flexibility leaving the area open to small-molecule peptides. The peptide must consist of two components: a helical peptide with a high binding affinity to PCSK9 and an appended extension with antagonistic properties to inhibit LDLR binding. The extension must encroach upon the LDLR binding site's hydrophobic pocket which significantly contributes to the binding energy of LDLR. Using two known PDB structures containing the removable peptides (A (5VLP) and B (6U3I)) in the N terminal grove, a three-dimensional printed model was created to demonstrate the interactions and proximity to the hydrophobic pocket. This model highlights critical amino acids on the peptides and PCSK9 to emphasize how the interactions support certain substitutions. One significant interaction lies between PCSK9 residues Ile369, Phe379, Asp238, and Ala239 and Peptide B's organic moiety (1-amino-phenylcyclohexane-1-carbonyl). These PCSK9 residues surround the LDLR binding region's hydrophobic pocket indicating a successful inhibition. On the other hand, Peptide A's FPG motif added to the Trp1 anchor formed a beta-turn which was unable to reach and, therefore, interact with PCSK9 residues Ile369, Phe379, Asp238, and Ala239. Through the 3D model, it was visualized that Peptide A had a beta-turn that precluded further extension into the target site, limiting its antagonistic ability. The model also highlighted Peptide B's attached organic moiety which reaches the hydrophobic pocket in the proximal LDLR binding region, as shown in the literature to increase the binding affinity by >100 fold with reduced overall mass for an improved oral therapeutic.

This work was made possible by funding through the National Science Foundation, Division of Undergraduate Education (NSF-DUE) grant number 1725940 for the CREST Project. Nova Southeastern University's Farquhar Honors College and Dept. of Biological Sciences also provided support. Protein model printing was made possible by 3d Molecular Designs.

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Abstract 1533

Towards Streamlined Custom Polyubiquitin Chain Synthesis

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Ubiquitin is a 76 amino acid protein that is added post-translationally to lysine residues on target protein substrates via an E1/E2/E3 enzyme cascade, thus impacting a wide array of protein-protein interactions, protein conformational changes, and cellular signaling cascades. Ubiquitin itself has 7 lysine residues and can be ubiquitinated at each of these sites, generating unique polyubiquitin chains that are implicated in diverse biological pathways. For example, K48-linked polyubiquitin is the canonical signal for protein degradation, while K63-linked polyubiquitin is implicated in the DNA damage response. With limitless combinations of potential linkage types and lengths available, discrete polyubiquitin chains are challenging to synthesize; current methodologies utilizing enzymatic chain extension lack the control to generate chains with user-specified linkage types and lengths, while chemical synthetic methods tend to be inefficient, time consuming, and lacking modularity. To streamline the process of custom polyubiquitin chain synthesis, we propose a chemically-controlled, enzymatically-driven, solid-phase synthetic approach. We couple the robust bacterial expression of ubiquitin, the control of protecting group chemistry, and the processivity of E1/E2 and Sortase enzymes to generate user-defined polyubiquitin chains of varying length and linkage type. We can attach these intact chains directly to solid-phase platforms enzymatically using Sortase, as well as attach monomeric ubiquitin to solid phase to then build the chains from the solid support. Leveraging some of the principles of solid phase peptide synthesis which enables high yielding, homogeneous, and facile generation of peptides, we employ alternating rounds of enzymatic chain extension and orthogonal deprotection steps to generate homogeneous custom polyubiquitin chains with ease.

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Abstract 1544

Ku70 binds the Epstein Barr virus protein BHRF1 in an acetylation-dependent manner

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The Epstein-Barr virus (EBV) affects more than 90% of the human population worldwide and is associated with infectious mononucleosis, cancerous malignancies, and multiple sclerosis. EBV counteracts apoptosis in infected cells by expressing BHRF1, a viral anti-apoptotic protein belonging to the BCL-2 protein family. The exact molecular mechanism that BHRF1 uses to stop apoptosis in infected cells remains to be elucidated. Ku70, a DNA repair protein, also plays roles in apoptosis through protein-protein interactions with BCL-2 and BAX, two members of the BCL-2 protein family. Based on the sequence and structural similarities between BAX, BCL-2, and BHRF1, we hypothesized that BHRF1 binds Ku70 and that this interaction may play important roles during EBV's evasion from apoptosis. Using Protein Fragment Fluorescence Complementation, we have detected a novel interaction between Ku70 and BHRF1. Since Ku70 acetylation can trigger apoptosis by preventing its binding with BAX, we are currently investigating whether the Ku70-BHRF1 interaction is also acetylation-dependent. Preliminary data suggest that acetylation mimetic mutations in Ku70 increase Ku70-BHRF1 interaction rather than prevent it. These results are consistent with a model where BHRF1 thwarts apoptosis in infected cells by binding acetylated Ku70 and preventing BAX release. To confirm this hypothesis, we are assessing the status of Ku70-BAX interaction in the presence of BHRF1. Our work may provide the basis to develop therapeutic strategies that may block infection and thus, reduce cancerous malignancies and other risks associated with EBV.

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Abstract 1545

Elucidating Iron Transport in the Periplasm of *Mycobacterium tuberculosis*

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The bacterium *Mycobacterium tuberculosis* (Mtb) causes the disease tuberculosis (TB) in humans, resulting in 10 million infections and 1.5 million deaths annually. Current TB treatment strategies involve a cocktail of up to 5 different antibiotics, many of which illicit harsh side effects. Furthermore, this treatment is usually administrated between 6 to 9 months. Due to this long and harsh treatment regimen, patient non-compliance is a major issue and has led to a rise in multiple-drug resistant strains of Mtb (MDR-TB). To combat these issues, novel TB treatment strategies are urgently needed. Host-acquired iron is essential for Mtb's survival, as such further elucidating Mtb iron uptake pathways could lead to identification of novel druggable targets. During infection, Mtb predominately acquires host iron via the siderophore-mediated iron uptake pathway, whereby small molecules with high affinity for iron are secreted to scavenge for host iron. We seek to shed light on the mechanism by which ferric-siderophores are transported across the Mtb periplasm and into the cytosol. At present there is little known about the proteins required to shuttle ferric-siderophores across the outer membrane, cell-wall environment, and periplasmic space to the inner membrane. Within the Mtb proteome, there are two putative Mtb periplasmic binding proteins (PBPs), FecB and FecB2, and we hypothesize that one or both of these PBPs may shuttle ferric-siderophores across the periplasmic space. To probe this hypothesis, we utilized tryptophan fluorescence quenching assays to determine the putative cargo of FecB and FecB2, as well as co-immunoprecipitation experiments in both *Mycobacterium smegmatis* and Mtb to identify potential interacting proteins *in vivo*. The data presented suggest that FecB plays a role in siderophore-mediated iron acquisition, and will be discussed in further detail. Finally, this study has broadened our understanding of the Mtb iron acquisition pathways and may lead to the identification of new therapeutic targets.

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Abstract 1547

Insight into the interaction between Tha4 oligomers and the other TAT components (Hcf106 and cpTatC)

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Most of the proteins that are required by chloroplast for the proper functioning in photosystems are encoded by genes in the nucleus and translated in the cytosol of plants. These newly synthesized proteins are transported into the chloroplast and to the thylakoids by the protein complexes assembled in the membranes. One pathway by which precursors are routed to the thylakoid lumen is by the chloroplast Twin Arginine Transport (cpTAT) pathway, which transports fully folded proteins by using proton motive force (PMF) as the only source of energy, across the thylakoid membrane. Precursor proteins targeted to the thylakoid contain a conserved twin-arginine motif (RR) in the N terminal targeting, or signal peptide, sequence. The cpTAT pathway consists of three membrane-bound proteins namely, Tha4, Hcf106, and cpTatC. Tha4 and Hcf106 share a similar structure with an N terminal transmembrane α-helix (TMH) followed by a short hinge region, an amphipathic helix (APH) and an unstructured carboxy-terminal C tail while cpTatC contains six linked transmembrane helices. From previous studies, it was found that the conserved glutamate residue (E10) in the transmembrane domain of Tha4 is essential for the function in the translocation of the precursor proteins and for the Tha4 assembly. It was found that the substitution of alanine for the glutamate prevents transport while an aspartate substitution partially recovers the transport. We predict that the E10 of Tha4 TMH acts as a sensor of the formation of PMF and aid the transport of the precursor. First, oligomer formation of each Tha4 E10/A/D variant, in the presence and absence of PMF was investigated by substituting cystines to the lumen proximate positions in the transmembrane helix of Tha4 by employing by radiolabeled crosslinking assays. Tha4 oligomer formation increases with an alanine substituted for the glutamate in TMH of Tha4, while an aspartate substitution showed a lower degree of oligomerization. From these studies, we show that modulations to helix hydrophobicity of Tha4 impact monomer and oligomer stability, and their packing thereby altering precursor transport via cpTAT pathway. Future studies will focus on studying the interaction between the Tha4 oligomers and other cpTAT components in the precursor complex.

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Abstract 1548

PRMT1 activates GATOR2-mediated mTORC1 signaling by methylating WDR24

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The mTORC1 pathway is a key sensor of a variety of environmental clues, such as growth factors, amino acids, and energy. By integrating these inputs, the mTORC1 pathway regulates many fundamental cellular processes, including lipid synthesis, glucose metabolism and autophagy, through which it controls cell growth and metabolism. Integrated proteomic analysis reveals that all components of the mTORC1 pathway undergo at least one type of posttranslational modifications (PTMs). However, the roles of these PTMs in mTORC1 activation remain large unknown. In this study, we employ a range of complementary methods, including biochemistry, molecular and cellular biology, functional cell-based assays, and xenograft mouse models to investigate how arginine methylation regulates mTORC1 signaling. We found that the protein arginine methyltransferase 1 (PRMT1) promotes R329 methylation of WDR24, an essential component of GATOR2, to enhance mTORC1 lysosomal recruitment and activation upon amino acid stimulation. High PRMT1 protein levels are associated with elevated mTORC1 signaling in specimens of liver cancer patients. Deficiency in WDR24-R329 methylation suppresses amino acid-induced mTORC1 signaling, cell proliferation and tumor growth. Our findings reveal an arginine methylation-dependent regulatory mechanism of mTORC1 activation and tumor growth and provides a molecular basis to target PRMT1 for cancer therapy.

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Abstract 1550

Structure and Mechanism of Zinc ABC Transporters in Bacteria

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ATP binding cassette (ABC) transporters are ubiquitous in all kingdoms of life, mediating the active transport of a vast array of substrates across the cell membrane. Minimally composed of an integral membrane permease and cytoplasmic ATPase, bacterial ABC importers also utilize an extracellular solute binding protein (SBP). The SBP is critical to the function of the transporter, binding the cognate substrate with high affinity and specificity and delivering it to the membrane permease for transport into the cytoplasm. The SBPs share a conserved structure where the substrate is bound between two structurally related alpha/beta domains. The structure of the interdomain linker classifies them into 7 clusters A-F with subdivisions based on substrate specificity. Cluster A-I SBPs mediate the transport of the essential transition metals iron, manganese, and zinc and are essential for virulence among many human pathogens. This makes them attractive drug targets, but their exploitation for this purpose will required detailed structural and mechanistic knowledge. Our objective is to determine the function, structure, and mechanism of bacterial zinc ABC transporters using various genetic, biochemical, spectroscopic and structural techniques including fluorescence spectroscopy, X-ray crystallography and NMR. Here we summarize our findings on the cluster A-I SBPs ZnuA and AztC as well as the recently identified zinc chaperone AztD, illustrating similarities and differences in their functions, structures, zinc binding properties, and dynamic behaviors. Progress on determining the permease and ATPase structures of these systems is also reported. Finally, we provide compelling evidence for the existence of an entirely new class of zinc SBPs and present the functional analysis, zinc binding properties and crystal structures of the representative protein ZrgA from *Vibrio cholerae*. The structure shows no similarity to cluster A-I SBPs and reveals several zinc binding sites not observed in the only other characterized homologue, PA4063 from *Pseudomonas aeruginosa*. The zrgA genes are conserved and associated with ABC transporter permease and ATPase genes in hundreds of bacterial species including many human pathogens. This family will have to be considered along with the classical cluster A-I ABC transporters when targeting bacterial zinc import for the development of novel antimicrobials.

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Abstract 1553

Structural and Regulatory Elements of Post-Translational Arginylation

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Eukaryotic post-translational arginylation, mediated by the family of enzymes known as the arginine transferases (ATE1s), is an important post-translational modification that can alter protein function and even dictate cellular protein half-life. Multiple major biological pathways are linked to the fidelity of this process, including neural development, cardiovascular development, cell division, and even the stress response. Despite this significance, the structural, mechanistic, and regulatory mechanisms that govern ATE1 function remain enigmatic. Research in my lab seeks to close this gap in understanding in order to target arginylation as a future therapeutic. While exploring arginine transferase function, we have discovered that ATE1s bind a previously undiscovered [Fe-S] cluster. We have used biochemical, spectroscopic, and analytical methods to decipher the composition and reactivity of this [Fe-S] cluster. Fascinatingly, we find that ATE1 cluster-binding preserves oligomeric homogeneity while increasing arginylation efficacy, demonstrating that this evolutionarily-conserved [Fe-S] cluster regulates arginylation rates. *In vivo* alterations of the [Fe-S] cluster-binding residues also compromise the ability of yeast (*Saccharomyces cerevisiae*) to respond appropriately to external stressors. Importantly, using a combination of X-ray crystallography, cryo-EM, and size-exclusion chromatography-coupled small angle X-ray scattering (SEC-SAXS), our lab has successfully solved the structure of *Saccharomyces cerevisiae* ATE1 (ScATE1). The three-dimensional structure of ScATE1 reveals a bilobed protein containing a canonical GCN5-related N-acetyltransferase (GNAT) fold. Structural superpositions and electrostatic analyses indicate this domain as the location of catalytic activity and tRNA binding. Additionally, our structure reveals the spatial connectivity of the N-terminal domain that binds the [Fe-S] cluster, hinting at the atomic-level details of the cluster's regulatory influence. Coupled with a new regulatory framework, the atomic-level structure of ATE1 brings us closer to answering pressing questions regarding the molecular-level mechanism of eukaryotic post-translational arginylation.

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Abstract 1561

Structure Analysis and Phenotype of the PTF1-J/Ptf1a P191 T Mutant Heterotrimeric Complex

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The Thomas Jefferson High School Biomedical Research Program in Dallas, Texas, studies the PTF1-J and PTF1-JL heterotrimeric complexes. The complexes are composed of a common E-box binding protein E12/47, Ptf1a and RbpJ or RbpJL. These complexes are transcriptional master regulators of pancreas development. PTF1-J is required for early pancreas development. During the secondary transition of pancreas development RbpJ is replaced by RpbJL. The PTF1-JL complex is required for the mature acinar cell phenotype. In the current study we have performed structural modeling using Pymol and Jmol of the PTF1-J and PTF1-J/Ptf1a P191T complexes. Children born without a functional Ptf1a protein lack a pancreas, are severely diabetic, require immediate medical intervention by administering insulin to maintain blood glucose homeostasis and or enzyme therapy to provide digestive enzyme activity in the small intestine for nutrient uptake into the bloodstream. In the current study we have performed structural modeling of the PTF1-JL/Ptf1a P191T complex and used 3-D printing technology to generate a model that may help explain the loss of function resulting in pancreatic aplasia in children born with this mutation. Determination of the structures for the PTF1-L and PTF1-L-Ptf1a(P191T) complexes will elucidate the mechanism for the strong cooperative binding of the PTF1-L complex to DNA and will provide insights into the structural changes that are introduced by the Ptf1a P191T substitution that cause pancreatic aplasia in children born with this mutation.

Junior League of Dallas Texas

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Abstract 1575

Towards the molecular mechanism of cotranslational nascent protein acetylation

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Denis Yudin, Nenad Ban, Shu-ou Shan

N-terminal acetylation is a ubiquitous protein modification that occurs on ~80% of the eukaryotic proteome. Depending on the protein substrate, N-terminal acetyl groups can mediate new protein-protein interactions resulting in changes to protein stability, subcellular targeting, and aggregation propensity, among others. Mutation and dysregulation of N-terminal acetyltransferases (NATs), which catalyze this reaction, have been linked to diseases such as cancer and Parkinson's. A large body of prior work suggests that most NATs bind to the ribosome and therefore act cotranslationally on newly synthesized polypeptides. However, previous biochemical measurements of NATs were performed with peptide substrates. While these studies provided high-resolution information about the structure of NATs and the chemical mechanism of N-terminal acetylation, it is unclear how NAT enzymes are recruited to elongating nascent proteins on the ribosome, or how they coordinate in space and time with other ribosome-associated protein biogenesis factors (RPBs) that compete for the nascent chain. Using purified ribosomes bearing nascent chains of defined sequence and lengths, we measured the binding of NatA/E to ribosome-nascent chain complexes (RNCs) and reconstituted the cotranslational acetylation of nascent proteins by these enzymes. Our initial results begin to define the timing of nascent protein acetylation during translation elongation and revealed a role of the nascent polypeptide-associated complex (NAC), an abundant and essential ribosome-associated chaperone, in mediating high-affinity NatA/E binding to ribosomes. Our finding parallels the recently discovered role of NAC in recruiting a cotranslational protein targeting machine, the signal recognition particle (SRP), to the ribosome. Thus, we posit that NAC may be a universal conductor at the polypeptide exit tunnel that coordinates the spatiotemporal recruitment of various ribosome protein biogenesis factors.

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Abstract 1607

Cardiovascular-Disease/Trait Associated Variants Alter NKX2-5 DNA Binding Affinity

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Transcription factors (TFs) are sequence-specific DNA-binding proteins that can bind to regulatory regions of the genome. Mutations in TFs have been proven to alter how these proteins recognize and interact with our DNA, leading to many diseases. For example, variants of NKX2-5, a cardiac TF essential for heart development, have been associated with congenital heart diseases (CHDs). However, mutations within the non-coding genome (e.g., promoters and enhancers) and their associations with diseases like CHDs, are less understood and present a novel area of research. Non-coding genomic variations can disrupt TF-DNA binding and alter gene regulation. Mutations predicted to impact NKX2-5 DNA binding were identified using SNP2TFBS, a position weight matrix (PWM) specificity predictive model. Using the ~84 million Single Nucleotide Polymorphisms (SNPs) from the 1000 Genomes Project, I identified 8,475 SNPs predicted to affect NKX2-5 binding. Of these, 901 SNPs occurred in putative cardiac enhancers, and 30 were disease-associated SNPs from the GWAS. The five variants with the largest predicted impact on TF-DNA binding affinity were tested *in vitro*: rs7350789, rs61216514, rs7719885, rs747334, and rs3892630. Four of these variants have been associated with cardiovascular disease or traits like cholesterol levels, hemoglobin traits, and systolic blood pressure. Recombinant NKX2-5 DNA-binding domain (DBD) was expressed using a bacterial expression system and purified through Ni-NTA affinity chromatography. Changes in NKX2-5 binding affinity were evaluated through Electrophoretic Mobility Shift Assay (EMSA) and quantified by calculating apparent dissociation constants (Kd) between variant and reference genomic sequences. We observed differential TF-DNA binding for all disease-associated variants. This project addresses the gap in knowledge on the molecular mechanism of non-coding variants associated with human diseases. These findings will aid in developing treatments for many diseases linked to mutations in the non-coding genome and early diagnosis through genetic testing.

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ASBMB

Abstract 1612

Assembly Checkpoint of the Proteasome is Mediated by Coordinated Actions of Proteasomal ATPase Chaperones

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Asrafun Nahar

The proteasome holoenzyme is responsible for degrading most proteins in the cell. In its 19-subunit regulatory particle (RP), a heterohexameric ATPase enables protein degradation by injecting protein substrates into the core peptidase. RP assembly utilizes "checkpoints", where multiple dedicated chaperones bind to specific ATPase subunits, and control the addition of other subunits. In the present study, we show that the RP assembly checkpoint utilizes two previously unknown features of the chaperones. Chaperones distinguish a defective RP, based on the nucleotide state of their cognate ATPases within it. Chaperones then modulate RP's ATP hydrolysis to facilitate subunit rearrangements for switching to active, substrate-processing states in the resulting proteasome holoenzyme. Specificity of these catalytic actions by the chaperones depends on their limited cellular pool, which is regulated by transcriptional repression by the NOT module of the Ccr4-Not complex. The RP assembly checkpoint serves as a potent regulator of protein degradation in the cell, impacting the fate of both proteasome holoenzyme and its protein substrates. Our findings provide a basis to potentially exploit the assembly checkpoints in situations with known deregulation of proteasomal ATPase chaperones.

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Abstract 1626

The mitochondrial Cu+ transporter PiC2 (SLC25A3) is a target of MTF1 and contributes to the development of skeletal muscle *in vitro*

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Copper (Cu) is a micronutrient necessary for the development of mammalian cells, specifically for skeletal muscle and neuronal tissues. Both tissues have an intrinsic high demand for Cu, as it is required for mitochondrial energy production among other functions. Cu transport to the mitochondria and targeting to mitochondrial cuproenzymes like Cu/Zn superoxide dismutase 1 (SOD1), and cytochrome c oxidase (COX) is necessary for the establishment of differentiated and functional cells. Disruption of mitochondrial Cu transport adversely affects growth and development, energy production, and can induce oxidative stress. Limited evidence is available regarding mitochondrial Cu-transporters that deliver Cu to mitochondria. The mitochondrial phosphate transporter hPiC2 is a candidate for delivery of Cu. In mammalian cells, there are two splice isoforms, PiC2A and PiC2B. PiC2A is expressed exclusively in cardiac and skeletal muscle, whereas PiC2B is ubiquitously expressed. In this work, we used primary myoblasts derived from mouse satellite cells to investigate the functional contributions of PiC2 to muscle growth and differentiation. CRISPR/Cas9 deletion of PiC2 impaired the expression of mitochondrial cuproproteins and delayed the growth and differentiation of myoblasts. Confocal microscopy and immunoprecipitation analyses showed that PiC2 interacts with several mitochondrial cuproenzymes. Importantly, PiC2 is a novel target of the metal-responsive transcription factor MTF1, which is required for skeletal muscle differentiation. Biochemical characterization of PiC2 showed that the transporter can bind Cu and that Cu transfer to COX is thermodynamically favored *in vitro*. Our work provides mechanistic information about a novel potential pathway for mitochondrial Cu transport and homeostasis in this organelle.

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Abstract 1628

Endothelial tip and stalk cell specification requires BMP9-induced βIV-spectrin expression during nascent vessel sprouting

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βIV-spectrin is a membrane cytoskeletal protein with emerging roles in the vascular system. Recent work demonstrates its critical requirement during sprouting angiogenesis, and data support that βIV-spectrin promotes stalk cell behavior by enhancing VEGFR2 turnover from the cell surface through a distinct phosphorylation dependent mechanism. Despite these fundamental roles in development, how βIV-spectrin itself is regulated remains largely unknown. Here we we report a novel crosstalk between BMP9 signaling and βIV-spectrin mediated VEGFR2 regulation. We identified BMP9 as a major inducer of βIV-spectrin gene expression. We found that, through Alk1/Smad1 pathway BMP9 markedly induces βIV-spectrin upregulation in both endothelial cells and in developing mouse retina which consequently limits VEGFR2 level and activity during angiogenesis. We also show that BMP9 upregulation of the canonical stalk cell genes including ID1/3 cannot compensate for the dysfunctional hypervascularity in βIV-spectrin-deficient mice due to the excessive VEGFR2 levels and tip cell accumulation. These findings suggest that the specification of tip and stalk cells strictly requires BMP9-induced βIV-spectrin expression during nascent vessel sprouting and we believe this study will be significant in broadening our understanding of the elusive spectrin biology in the vascular system.

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Abstract 1641

Designing a FGF1-FGF21 chimeric protein with enhanced activities

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The fibroblast growth factor (FGF) family is consisted of 22 proteins that mediates various biological functions: from wound healing, tissue repairs, to angiogenesis and glucose regulations. There are three main modes of action within FGFs: paracrine, intracrine or endocrine. FGF1 is one of the paracrine FGFs that works locally through interaction with FGF receptors and heparin sulfate (HS) to regulate developmental processes: cell growth and differentiation, tissue repairs, and tumor growth and invasion. The FGF1 has been studied extensively and led to the construction of a variant termed SuperFGF1. SuperFGF1 exhibits increased thermal, chemical, and proteolytic stability, enhanced bioactivity, and reduced HS binding affinity. FGF21 is an endocrine FGFs that participates in long range physiological processes but does not require HS. It engages in a plethora of regulations across various organs. The most notable activity of FGF21 is its mediation of glucose uptake in adipocytes. Thus, FGF21 is an attractive therapeutic agent for metabolic diseases such as Type-2 Diabetes. However, FGF21 possesses an inherently unstable core with low thermal stability and receptor affinity. Herein, we designed a structure-based chimera protein by fusing the physiologically active FGF21 C-terminal with a thermally stable FGF1 variant. Using circular dichroism spectroscopy, we show that the chimera protein sFGF1-FGF21 adopts a α-helix motif. Intrinsic fluorescence spectra of the chimera protein shows an emission maximum centered at 345 nm indicating that the emission of the conserved tryptophan is not quenched, similar to wildtype FGF21. Differential calorimetry data suggest that chimera sFGF1-FGF21 is significantly more stable than wild type FGF21. Trypsin digestion assay indicates that the chimera is prone to proteolytic cleavage, similar to FGF21. Cell proliferation assay with NIH3T3 showed that the chimera protein has strong proliferative activity. The findings of this study provide valuable clues for the rational design of FGF21-based therapeutic against metabolic diseases.

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ASBMB

Abstract 1654

ORF28 of HHV-8 interacts with the E3 CHIP to mediate the ubiquitination of host membrane proteins

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Carboxyl-terminus of Hsp70-Interacting Protein (CHIP) is an E3 ubiquitin ligase that is canonically known to mark misfolded chaperone clients for proteasomal degradation. Recent publications, including our own work, indicate CHIP has a previously underappreciated role in chaperone-independent ubiquitination of substrates and a multitude of Hsp-independent interactors were predicted based on CHIP's binding motif (PMID: 31320752). One predicted high-affinity interactor is ORF28, an under-characterized human herpesvirus 8 (HHV-8) membrane protein. We demonstrate CHIP associates with ORF28, and ORF28 expression causes CHIP to relocalize to the cell membrane. Host ubiquitination substrates were identified via proteomics, suggesting this interaction enables remodeling of the cell surface proteome during viral infection. While similar in principle and function to the viral E3 ubiquitin ligases K3/K5, this manipulation of CHIP by ORF28 to ubiquitinate host proteins may represent a novel mechanism of immune evasion in HHV-8.

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Abstract 1658

Haemophilus influenzae Protein D antibody suppression in a multi-component vaccine formulation

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Haemophilus influenzae (NTHi) is a commensal bacterium in the nasopharynx, but can turn pathogenic under certain circumstances, causing Acute Otitis Media (AOM) in children, acute sinusitis in children and adults, and acute exacerbation of chronic bronchitis in adults. To protect against NTHi infection, a multi-component protein vaccine would be advantageous for broad coverage and to avoid the emergence of vaccine-resistant strains. A few leading protein vaccine candidates against NTHi are Protein D (PD), outer membrane protein (OMP) 26, and Protein 6 (P6). All three of these proteins induced strong antibody responses in mice as individual vaccines, but when PD and OMP26 were combined into a single vaccine formulation, PD antibody levels were significantly suppressed. We hypothesized that OMP26 and PD interacted physiochemically to mask the PD antigenic response. However, results from *in vitro* mass spectrometry and column chromatography experiments did not support this hypothesis. We then hypothesized that PD antibody suppression occurs *in vivo* through antigenic competition with OMP26. In support of this hypothesis, we showed that PD and OMP26 injected into separate legs of the mouse induced a PD antibody response similar to that of individual PD vaccination levels. We conclude that mixing PD and OMP26 into a single vaccine formulation will require further investigation.

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Abstract 1663

Extracellular protease calpain cleaves Kv1.5 at the S1-S2 linker and increases the channel current

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The voltage-gated potassium channel Kv1.5 plays important roles in the repolarization of atrial action potentials and regulation of the vascular tone. We have previously shown that the Kv1.5 channel is cleaved by a fungal enzyme Proteinase K (PK), which cleaves the mature, cell-surface Kv1.5 protein, leading to an increase in the Kv1.5 current (IKv1.5). The objective of the present study is to investigate the effects of physiologically relevant proteases on Kv1.5 channels. Mutagenesis, patch clamp, Western blot, and immunocytochemical techniques were used to study the function, expression, and cellular localization of Kv1.5 channels upon protease treatments. Our results showed that culturing of Kv1.5 expressing HEK cells with white blood cells (WBCs) for 12 h led to a decrease in mature channel expression and an increase in IKv1.5. The cysteine protease calpain, that is upregulated in inflammatory conditions and is secreted by WBCs, was detected in the culture medium with WBCs, and calpain inhibitor abolished WBC-mediated increase in IKv1.5. Application of medium containing calpain cleaved mature Kv1.5 protein and resulted in an increased IKv1.5. Based on the size of the fragments resulting from the cleavage, we identified a potential cut region located between residues 282–300 in the extracellularly exposed S1-S2 linker of the channel. Deletion of residues 282–300 abolished calpain-mediated cleavage and the associated IKv1.5 increase. Since extracellular proteases such as calpain may be increased under inflammatory conditions in the heart and blood vessels, our findings offer new insights to ion channel regulation and its pathophysiological implications.

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Abstract 1669

Studying the Effect of Relevant Mutations on γB Crystallin Structure and Dynamics

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Crystallins are the predominant structural proteins in the lens of the eye. The dysfunction of specific inter-protein interactions between the crystallins is known to cause the proteins to aggregate and phase-separate, causing the lens to move from clear to cloudy. Posttranslational mutations, some of which are the result of aging and UV damage, can lead to changes in the chemical and physical properties of the proteins, thus affecting inter-protein crystallin interactions and resulting in cloudiness called cataracts. Studying biologically relevant mutants of crystallin proteins, in comparison with wild-type (non-mutated) crystallin, will provide more information on how specific residues dictate the detailed interactions between proteins, which can lead to cataracts. Here, we describe our work with clinically and/or biologically relevant site-directed mutants of bovine γB crystallin, homologue to human γD crystallin (Eg. D107A and W42R). Alongside other biochemical and biophysical experiments, we used nuclear magnetic resonance (NMR) spectroscopy to determine how the mutations affected the interactions between the γB crystallin proteins. Preliminary results suggest that chemical shifts determined using NMR are highly sensitive to changes in intermolecular interactions. These studies will add to our understanding of how mutations in crystallins affect inter-protein interactions, protein aggregation, and cataract formation.

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Abstract 1670

The crystal structure of a PTE RNA element from a cactus virus that binds human eIF4E

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The 3′ untranslated regions of the tombusvirus genomes contain cap-independent translation enhancers (CITEs), which promote genome translation through cap-independent mechanisms. These structures are known to bind translation initiation factors or the ribosomal subunits. They have been proposed to interact with the 5′-end to circularize the viral genome, thus priming the genome for translation in the absence of 5′-cap. However, except for a few computational models and secondary structures determined from biochemical studies, there are no high-resolution crystal structures for the various classes of 3′-CITEs. Here, we have determined the crystal structure of a PTE (Panicum Mosaic Virus-like 3′-CITE) from the Saguaro Cactus Virus (SCV) genome in a complex with a Fab chaperone. The SCV PTE folds into a T-shaped three-way junction with a pseudoknot formation between the pyrimidine-rich region within the junction and the purine-rich bulge within the first stem. The bulge flips out a G18 nucleotide to create a solvent-exposed binding site for eIF4E, consistent with previous biochemical probing studies. The crystal structures of G18C and G18A mutants that crystallize under identical conditions are very similar to the wild-type structure, suggesting that SCV PTE pre-organizes its bulge structure, forcing the G18 nucleotide to flip out. Further studies with the human and wheat eIF4E using isothermal titration calorimetry (ITC) assays show that both human and wheat eIF4E bind with the SCV PTE constructs, suggesting that the PTE perhaps mimics the mRNA 5′-cap for recruiting the eIF4E and initiating the viral genome translation.

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Abstract 1677

Elucidating Microbial transglutaminase 2 and the role of Polyproline II Structural Motif in Celiac's Disease

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Microbial transglutaminase 2 (mTG2) is an enzyme used ubiquitously throughout the food industry to primarily improve the texture of protein based food products. Moreover, mTG2 can also react with gliadin peptides from several cereals, including wheat. Celiac's Disease (CD) is an autoimmune disorder characterized by increased intestinal permeability and gastroenteritis symptoms due to a reaction to gliadin peptides found in gluten. The human transglutaminase 2 (TG2) is responsible for the deamidation of glutamate. Deamidation of gliadin peptides results in their adoption of a polyproline II structures, in turn increasing their binding affinity to human leukocyte antigen (HLA) DQ2 and DQ8. This interaction triggers a strong immune response associated with the symptoms of CD. It has been previously demonstrated that mTG2 can turn gliadin protein immunogenic like TG2. Polyproline II structure is typical of peptides bound to major histocompatibility complex II proteins, implicating this structural motif in gluten intolerance. Additionally, the immunogenic 33-mer gliadin peptide and its homologs have been shown to have a strong type II polyproline helical character when deamidated via far UV circular dichroism (Far UV-CD). Here we are testing the ability of gliadin peptides deamidated by mTG2 which adopt a type II poly proline structural motif to increase intestinal permeability using a Zebra fish model.

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Abstract 1694

Do post-translational modifications of mitochondrial RNA polymerase and ribosomal protein L12 control mitochondrial DNA transcription?

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Mitochondria play an important role in energy production and cellular metabolism. Mitochondria contain their own DNA (mtDNA), which encodes 13 subunits necessary for oxidative phosphorylation. Over 1500 other mitochondrial proteins, including the 77 remaining oxidative phosphorylation subunits and the machinery required for transcription and translation of mtDNA, are encoded by the nuclear genome. Thus, transcription of the nuclear and mitochondrial genomes must occur in concert to respond to the energetic needs of the cell. The mechanism of this communication is unclear and transcriptional regulation in the mitochondria is not well understood. The mitochondrial proteome, including the transcriptional machinery, is subject to post-translational modifications (PTMs) such as phosphorylation of serine and threonine, and acylation of lysine. We hypothesize that PTMs of the mitochondrial transcriptional machinery regulate mitochondrial gene expression, akin to mechanisms controlling nuclear gene expression. Transcription of mtDNA requires three nuclear-encoded proteins: mitochondrial transcription factor A (TFAM), transcription factor B2 (TFB2M), and mitochondrial RNA polymerase (POLRMT). Mitochondrial ribosomal protein L12 (MRPL12), an accessory factor, is thought to stabilize POLRMT and promote transcription. Using mass spectrometry, POLRMT and MRPL12 were shown to contain numerous sites of acetylation and phosphorylation, including sites that are not currently documented in literature. The biochemical function of these modifications is unknown. PTMs were studied using site-directed mutagenesis to replace the amino acid of interest and mimic acetylation (lysine to glutamine) and phosphorylation (threonine to glutamate) of POLRMT or MRPL12. Mutated proteins were purified by affinity and ion exchange chromatography. Their binding affinity for the mtDNA promoter was determined by fluorescence anisotropy experiments. These experiments revealed that POLRMT PTM mimics had little effect on mtDNA binding. In addition, when MRPL12 and MRPL12 PTM mimics were co-incubated with WT POLRMT, mtDNA binding affinity was largely unaffected. Efforts are ongoing to characterize additional PTM mimics and to assess the stability of POLRMT in the presence of MRPL12 PTM mimics.

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Abstract 1698

Biophysical Characterization of the LATS1-YAP Interaction in Hippo Signaling

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Cellular level mediation between proliferation (cell growth) and apoptosis (programmed cell death) are integral factors in promoting the healthy development of organisms. Originally discovered in Drosophila, Hippo signaling is an evolutionarily conserved signaling pathway that modulates organ size and tissue homeostasis through global regulation of cell proliferation and apoptosis. Dysregulation of the Hippo signaling network gives rise to uncontrolled cell growth and the onset of pathological conditions, notably hyperplasia and tumorigenesis. Post-translational modifications regulate the subcellular localization, activity, and stability of proteins in Hippo signaling. For instance, when Hippo signaling is activated in mammals, the transcriptional co-activator YAP (Yes-associated protein), the terminal effector of the Hippo pathway, is phosphorylated by Large Tumor Suppressor Kinase 1 (LATS1). Phosphorylated YAP is sequestered in the cytosol in protein-protein regulatory complexes or ubiquitinated and degraded, where it is no longer able to facilitate the transcription of genes promoting cell growth and proliferation. In turn, LATS1 kinase activity is also regulated by phosphorylation at a key threonine residue (T1079), however, it is unclear how this post-translational modification modulates the stability of the LATS1-YAP interaction. In the work herein, we use isothermal titration calorimetry to investigate the energetics of binding of YAP to unphosphorylated (WT-LATS1), and a phosphomimetic (LATS1 T1079D) of LATS1. Our experimental results resolve the question of how LATS1 phosphorylation modulates its interaction with YAP1. We find that phosphorylation does not cause significant changes in the stability of the complexes, and although phosphorylation may not modulate the strength of the interaction, it brings about significant changes in the binding energetics that could be finetuned to regulate.

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ASBMB

Abstract 1699

Biochemical characterization of CRSP2, a novel Cu-binding protein implicated in the development of cultured myoblasts

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Copper (Cu) is a vital micronutrient that is imperative for the proliferation and development of mammalian cells. Surplus of Cu is toxic to cells, so a broad network of transporters, chaperones, and regulators maintains the homeostasis of Cu. Failure in the homeostatic machinery of Cu confers various muscular degradation pathologies such as Menke's and Wilson's disease. The Metal Regulatory Transcription Factor 1 (MTF1) is a key regulator of the cellular Cu networkthat ensures metal homeostasis. In addition, we found that MTF1 is also necessary for the differentiation of primary myoblasts derived from mouse satellite cells. MTF1 does not act alone, immunoprecipitation assays coupled to mass spectrometry, suggested that MTF1 interacts with chromatin remodeling proteins and a novel group of Cu-binding proteins present in myoblasts. CSRP2 has emerged as a novel Cu-binding protein with redox potential determined by cyclic voltammetry analyses among these interactors. Recombinant purified CSRP2 protein binds at least two Cu+ which confers the redox potential to the protein. We are characterizing additional biochemical properties of CSRP2, as a potential component of a novel Cu-dependent regulatory network which orchestrates metal homeostasis and the development of skeletal muscles *in vitro*, which represent potential targets for the treatments of Cu-related myopathies and muscular phenotypes in patients with Menkes or Wilson's diseases.

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Abstract 1705

TWIST1 Basic Domain Mutants retain DNA binding activity to a conserved E-box from the TNF α gene

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Noe Crespo-Hernandez, Jessicca Renta-Torres, Carmen Cadilla-Vazquez

TWIST1 is a basic Helix-loop-Helix transcription factor important in craniofacial development. Mutations in the basic domain of TWIST, responsible for DNA binding, have been shown to cause Saethre-Chotzen and related disorders as well as Sweeney-Cox syndrome. These syndromes are characterized mainly by skull deformities and facial dysostosis. Missense mutations in three highly conserved residues in the basic domain, two arginines and one glutamate, have been associated with defects on the head and facial structures as well as limb abnormalities. We hypothesize that mutations in the basic domain of TWIST1 disrupt its DNA binding activity, leading to affinity and specificity changes causing gene dysregulation in Saethre–Chotzen and Sweeney-Cox and other syndromes. TWIST1 mutants were produced by site directed mutagenesis, expressed in BL21 *E. coli* cells, and purified by affinity and ion exchange chromatography. Protein yields ranged from 1.76 to 0.18 mg/L of culture. To assess the DNA binding activity, Electrophoretic Mobility Shift Assays (EMSA) were performed using an infrared labeled oligonucleotide containing a conserved E-box in the TNFα promoter. Wild type TWIST1 and the assessed mutants, R116L, E117G, E117V and R118H showed DNA binding activity. Biolayer Interferometry analysis showed that E117 and R118H mutants had a larger kd compared to the wildtype protein, indicating rapid dissociation from the TNFα E-Box. For these mutants, changes in sequence specificity and/or dominant negative effects could explain the phenotype observed in these autosomal dominant syndromes.

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Abstract 1718

Differential effects of remdesivir and lumacaftor on homo- and heterotetrameric hERG channels

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Shetuan Zhang, Wentao Li, Jun Guo, Tonghua Yang

The human ether-a-go-go-related gene (hERG) encodes for the pore-forming α-subunit of the channel that conducts the rapidly activating delayed K+ current (IKr) in the heart. hERG channel is important for cardiac repolarization, and its reduction due to loss-of-function mutations causes long-QT syndrome type 2 (LQT2), a cardiac condition with high risk of arrhythmias and sudden death. Most LQT2 mutations impede hERG trafficking to the plasma membrane, and promotion of hERG channel trafficking is a strategy to rescue mutant channel function. The purpose of this study is to study drugs that can rescue the function of mutant hERG channels. The methodological techniques used in this study were patch clamp, Western blot, and immunocytochemistry. As we recently found that the antiviral drug remdesivir increases wild-type (WT) hERG current and surface expression, we studied the effects of remdesivir on trafficking deficient LQT2-causing hERG mutants G601S and R582C. We also investigated the effects of lumacaftor, a drug used to treat cystic fibrosis that promotes CFTR protein trafficking and has been shown to rescue membrane expression of some hERG mutations. Our results show that remdesivir or lumacaftor did not rescue the current and cell-surface expression of homotetrameric mutants G601S and R582C. However, remdesivir decreased and lumacaftor increased the current and cell-surface expression of heterotetrameric channels formed by co-expression of WT hERG with mutant G601S or R582C hERG. These findings that drugs can affect homotetrameric WT and heterotetrameric WT+G601S (or R582C) differently are novel and have clinical implications for patients with hERG mutations.

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Abstract 1719

Activation of human small heat shock protein HSPB5 by zinc: Taking advantage of a multitude of possibilities

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Rachel Klevit

Small heat shock proteins (sHsp) are a cell's first stress response units. They function to delay protein aggregation, implying that sHsps are resistant to cellular stress conditions and may instead be activated by them. Indeed, stress factors known to lead to activation of sHsp chaperone activity include acidosis, and changes in metal ion concentration. Here we expand upon previous findings from our and other laboratories on the ubiquitously expressed human small heat shock protein HSPB5 (aka, αB-crystallin) which showed that 1) a highly conserved histidine residue plays an important role in HSPB5 activation and 2) metal ions can activate and cause structural rearrangements in HSPB5. We sought to determine in detail the effects of zinc on HSPB5. HSPB5 forms polydisperse and interconverting ensembles of large oligomers. Despite ∼50% of HSPB5 sequence space being intrinsically disordered, it contains numerous conserved histidine residues: of the 9 histidines in its sequence, four are in the disordered region (NTR domain) and 5 are in the stably folded core domain (ACD domain). Our isothermal calorimetry data reveal 2 macroscopic zinc-binding constants. Paradoxically, although histidines in the structured ACD domain have previously been implicated in metal-ion binding, we observe a higher affinity binding that involves the disordered NTR. Furthermore, 9 single histidine-to-alanine mutations revealed that no single histidine is critical for either the strong or weak binding events. Such behavior implies remarkable plasticity in zinc binding and a role for disordered regions in zinc binding in HSPB5. We also observe a substantial Zn2+-dependent decrease in subunit exchange between oligomers and evidence of conformational changes in the oligomer, implying inter-subunit Zn2+ binding within oligomers. We propose a model for the observed metal ion plasticity of HSPB5 in which binding sites are not pre-defined in the absence of metal ions and there exist multiple ways in which metal binding can be executed. Our findings shed light on the modes of action of HSPB5 in preventing protein aggregation by identifying plasticity as a key factor in zinc binding. Taking these findings into a bigger picture, metal misbalance can have calamitous effect on cells and induce protein aggregation. Prolonged exposure to metals is implicated in the development of debilitating diseases, thus understanding the modes and pathways cells utilize to prevent it is of crucial importance.

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Abstract 1725

Investigating the molecular structure and function of the human HOPS complex

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Autophagy is an evolutionarily conserved degradation pathway that packages and delivers malfunctioning or unnecessary components to the cellular incinerator (lysosome) for breakdown and recycling. Although much is known about the mechanism underlying cargo selection and autophagosome biogenesis, the mechanisms of how the autophagosome is specifically delivered to, and efficiently fuses with, the lysosome are not fully understood in human biology. The HOPS (homotypic fusion and vacuole protein sorting) complex is a ~580 kDa, 6-subunit assembly that plays essential roles in mediating membrane fusion events in both late endosome trafficking and autophagy. Previous studies on yeast HOPS suggested that this complex serves as a tethering factor, linking incoming transport vesicles to a target organelle and mediating membrane fusion via complex formation between membrane-bound opposing SNARE proteins (soluble N-ethylmale-imide-sensitive factor-attachment protein receptors). Indicative of the high degree of difference between the human and yeast autophagic environments, very little is known about the human HOPS complex due primarily to technical difficulty in its isolation. To address this problem and associated knowledge gaps, we have developed a baculovirus-insect cell-based system to produce and reconstitute human HOPS. Using mass spectrometry and mass photometry, we were able to assess the composition and integrity of purified recombinant human HOPS complex. Our single-particle negative stain electron microscopy (EM) analysis demonstrated an overall elongated fibrillar architecture in human HOPS, reminiscent of yeast HOPS structural studies and an in-silico model generated by ColabFold. Lastly, we found that human HOPS, like its yeast counterparts, binds strongly to the autophagosome associated SNARE protein syntaxin17. In summary, we have established a biochemical platform for characterizing the structure and function of the enigmatic human HOPS, thereby allowing us to delineate the similarities and differences between human and yeast HOPS.

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Abstract 1734

Investigating the Interactions of the human Autophagy Factor and Vici Syndrome Protein EPG5

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Autophagy is an evolutionarily conserved degradation system for maintaining cellular homeostasis. This system sequesters cytoplasmic material into a transport vesicle termed the autophagosome and delivers this cargo-laden "package" to the lysosome for breakdown and recycling. This multi-step pathway is orchestrated by a specialized machinery composed of different Atg (autophagy related) and non-Atg proteins. Although previous studies on different Atg proteins have advanced the knowledge on the early steps of autophagy, the later phases of autophagy remain poorly defined. Originally identified in the *Caenorhabditis elegans* genetic screen, EPG5 (ectopic P granules protein 5) is a metazoan-specific autophagy factor that is thought to mediate autophagosome-lysosome fusion. Recessive mutations of EPG5 results in accumulation of autophagosomes in cells and causes a severe multi-system disorder in humans known as Vici syndrome. Recent studies have shown that human EPG5 (hEPG5) can bind to members of the LC3/GABARAP family of autophagosome proteins. More specifically, hEPG5 contains two LC3-interacting region (LIR) motifs (hLIR1 and hLIR2) for LC3/GABARAP binding. To gain further insights into mechanism-of-action of this important autophagy factor and its interactions with the LC3/GABARAP family proteins, we are applying both biochemical and structural approaches to characterize such interactions. *In vitro* pulldown experiments showed that hEPG5 preferentially interacts with the GABARAP members of the LC3/GABARAP family which have previously been shown to mediate later stages of autophagy. Using a combination of *in vitro* pulldown and isothermal titration calorimetry (ITC), we showed that hLIR2 motif binds with higher binding affinity to the GABARAP proteins than the hLIR1 motif. Nuclear magnetic resonance titration experiments demonstrated that hLIR1, hLIR2 and the tandem hLIR1-hLIR2 motifs interact with GABARAPL1 in a similar fashion. Crystallographic analysis further confirmed that hLIR2 motif binds to the canonical LIR motif-binding site on GABARAPL1. Based on these results, we proposed a step-wise binding model for hEPG5-GABARAP interaction: hLIR1 serves as an anchoring motif for making initial contact with GABARAP at the canonical binding site, which then triggers a local conformational change and allows hLIR2 to displace hLIR1 at the binding site.

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Abstract 1738

Analysis of structural similarities between TAM receptors and other cell surface molecules

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TAM receptors represent a unique non-growth factor associated receptor subfamily of receptor tyrosine kinases (RTKs). These receptors have a vital role in maintaining cellular homeostasis through the clearance of apoptotic cells and control of inflammatory and immune responses. Linked to these important regulatory roles, dysregulation of TAM receptors is implicated in numerous disease states, but little is currently known about the structural changes that are associated with their activation and that underly their role in these various diseases. Furthermore, while there is growing interest in TAM receptors as therapeutic targets, their multiple roles in homeostatic processes create challenges for developing therapeutic strategies. Understanding TAM receptor activation mechanisms is important for further investigation of the potential development of targeted therapies. While these receptors are commonly believed to be activated through classical receptor-induced dimerization, there are no current studies that confirm this activation mechanism applies for TAM receptors and preliminary work suggests alternative mechanisms may be possible. My work utilizes a combination of structural, biophysical and biochemical approaches to investigate TAM oligomerization and cross-talk with other receptors, and here I will present early structural analysis of TAM receptors and intriguing similarities to cell-cell adhesion molecules and receptors that are involved in higher order oligomerization or clustering. Furthermore, my presentation will introduce some of the future goals of my new lab, which officially opens in February of 2023.

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Abstract 1754

Can Aged-Damaged Proteins Be Targeted for Degradation? Early Insights in the Structural and Molecular Characterization of Human Protein-L-Isoaspartate O-Methyltransferase Domain-Containing Protein 1 (PCMTD1)

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L-asparagine and L-aspartate residues can non-enzymatically isomerize into L-isoaspartate residues which can contribute to the decreased functionality of proteins. Because these reactions are spontaneous, the accrual of this damage naturally increases as one ages. Historically, the methylation-induced repair activity initiated by protein-L-isoaspartyl (D-aspartyl) O-methyltransferase (PCMT1) was thought to be the only cellular mechanism which combats the accrual of L-isoaspartyl damages. However, recent studies suggest an additional mechanism may exist in which L-isoaspartyl damaged proteins may be preferentially recognized and degraded through the ubiquitin-proteasomal system. Current work suggests protein carboxyl methyltransferase domain-containing protein 1 (PCMTD1) is a potential E3 ubiquitin ligase that ubiquitylates proteins harboring isoaspartyl damages for proteasomal degradation. Similar to PCMT1, the N-terminal domain of PCMTD1 contains L-isoaspartate and S-adenosylmethionine (AdoMet) binding motifs needed for isoaspartyl repair activity. This protein also contains SOCS-box recruitment motifs found in substrate receptor proteins which eventually complex into active multimeric cullin-RING E3 ubiquitin ligases (CRLs). While PCMTD1 is able to bind to the canonical methyltransferase cofactor AdoMet, isoaspartyl repair activity has not yet been demonstrated by PCMTD1. However, PCMTD1 is able to associate with components of the CRL system, Cul5 and Elongins B and C, *in vitro* and in cells. Early work in negative stain electron microscopy and native top-down mass spectrometry to characterize the structural dynamics of PCMTD1 further suggests PCMTD1 multimerizes with CRL components to form the putative E3 ubiquitin ligase, CRL5-PCMTD1. While further molecular and structural characterization of PCMTD1 is needed to better understand this newly proposed preotolytic pathway for aged-damaged proteins and the potential structure-function relationship of PCMTD1, we describe here initial studies of this previously uncharacterized protein which may ultimately function as an isoaspartyl-residue-specific E3 ubiquitin ligase.

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ASBMB

Abstract 1763

The Identification of Mutations Promoting Amyloidogenic Transitions (IMPAcT) method for identification of previously unrecognized amyloid disease-related proteins

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Low-complexity domains (LCDs) of proteins have been shown to self-associate, and pathogenic mutations within these domains often drive the proteins into amyloid aggregation associated with disease. Such disease-related LCDs are found in proteins TDP-43, FUS, hnRNPA2, among others. We developed the IMPAcT method to search for pathogenic mutations in LCDs of the human proteome which induce amyloid aggregation, leading to identification of other proteins associated with amyloid disease. We calculated the amyloid propensity of each relevant LCD protein segment with the wild-type and variant sequence to identify those mutations which are most likely to promote the amyloid aggregation of an otherwise functional protein. Biochemical and structural analysis of candidate proteins successfully confirmed two novel amyloidogenic proteins, protein TFG and keratin-8, based on multiple amyloid-promoting pathogenic mutations within each protein. Based on this evidence, we hypothesize amyloidosis to be a component of the etiology of the associated diseases. Further characterization of other proteins identified by the IMPAcT method may reveal even more novel amyloid proteins.

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Abstract 1764

Using the photo-crosslinkable non-canonical amino acid BZF to capture U.V. driven, state-dependent disruption of kinetics and voltage-dependence of activation in hERG potassium channels

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Gail Robertson, Matt Trudeau

Human ether-á-go-go related gene (hERG) is a voltage-gated potassium channel that is critical for cardiac excitability. The characteristic slow closing (deactivation) of hERG is regulated by a direct interaction between the cytosolic N-terminal Per-Arnt-Sim (PAS) domain and the C-terminal cyclic nucleotide binding homology domain (CNBHD), and this slow closing plays a role in repolarizing the cardiac action potential. Here we aim to understand if the conformation of the PAS domain is coupled to the transmembrane regions of the channel that harbor the voltage sensor domain and channel gate, to contribute to the voltage sensitivity and gating kinetics of the channel. To achieve this, we genetically incorporated the photo-activatable ultraviolet (U.V.) cross-linkable non-canonical amino acid 4-Benzoyl-L-phenylalanine (BZF) using TAG codon suppression directly into the PAS domain of hERG. BZF in this application serves as a small non-perturbing chemical probe that when U.V. irradiated can form a covalent cross-link with C-H bond-containing groups within C-C bond length proximity. With BZF incorporated into the PAS domain of hERG we U.V. irradiated channels at discrete holding voltages using excised inside out patch clamp-electrophysiology. That hERG is a voltage-gated ion channel, we hypothesized that irradiation of the channel at discrete voltages would capture state-dependent effects if the PAS was changing conformation during channel gating. In fact, we observed a potent U.V. photo-driven and state-dependent change in the biophysical properties of the channel. When compared to wild-type hERG1a, hERG1a-BZF showed a U.V. dose-dependent change (speeding up) of channel deactivation when irradiated in the closed state at −100 mV holding voltage. Additionally, hERG1a-BZF exhibited a U.V. dose-dependent and marked change (right-shift) in the voltage-dependence of activation when irradiated at −100 mV when the channels were closed. These effects were not observed when the hERG1a-BZF channel was irradiated at 0 mV when the channels were open. This approach demonstrates that direct photo-crosslinking of hERG channels causes a measurable change in biophysical parameters and this effect is state-dependent (occurring here in the closed state but not the open state). We propose that altered channel gating is as a direct result of reduced dynamic motions in the hERG channel due to photo-chemical crosslinking and that PAS domain conformational changes are coupled to channel gating and voltage dependence.

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Abstract 1769

Preserving Human Milk Proteins: Ultraviolet light as an alternative pasteurization method

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Human milk is a dynamic fluid that can meet the nutritional needs of infants while sustaining their immunity and promoting their growth, development, and metabolism. The complex makeup of milk consists of bioactive proteins, hormones, and growth factors that contribute to the well-being of the infant. Mother's own milk is the preferred option for newborns, especially premature neonates. Preterm infants are typically immunocompromised and experience a higher likelihood of mortality and mobility, and milk can support their protection against illnesses. When mothers cannot produce milk, or a sufficient amount, it can be supplied through Human Milk Banks (HMB). These facilities currently use the standardized Holder pasteurization (HoP) methods to purify milk from pathogens that could potentially severely impact the infant's health. However, the conditions of HoP (62.5C, for 30 min) requires a heat treatment that is known to substantially reduce the abundance of functional compounds in human milk. Therefore, non-thermal alternative pasteurization processes including Ultraviolet light (UV-C) have promising opportunities to maximize the number of beneficial compounds in human milk while still decreasing harmful pathogens by 5-log. In this research study, human milk was first processed under a variety of UV-C (1,000 J/L −18,000 J/L) conditions to identify the optimal treatments for maintaining the natural structure and function of milk compounds. Milk treatments were then analyzed using activity and enzyme-linked immunoassay to understand specific protein abundance and their concentrations compared to UV-C, HoP, and raw milk samples. The findings thus far, demonstrate that UV-C did not significantly affect the concentration of plasmin, elastase, cathespin D, and glutathione peroxidase compared to HoP in human milk. UV-C is a promising alternative non-thermal novel technology for improving the safety of human donor milk. UV-C preserves a larger array of bioactive proteins (e.g lactoferrin, lysozyme, bile-salt stimulated lipase) in human milk to a greater extent than does HoP. This information will enable donor milk processors and clinicians to select the optimal processing methods to preserve specific milk proteins and bioactivities.

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Abstract 1774

Understanding the interaction between the HPV E2 protein and its binding site variants

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Subhasis Biswas

The human papillomavirus (HPV) is the most common sexually transmitted diseases, according to the Centers for Disease Control. There are over 100 HPV strains that can be divided into two groups based on their oncogenic potential: low risk and high risk (carcinogenic). Despite this, there are still no treatments or cures for people with existing HPV infections. Therefore, there is a need to further investigate this virus and find a potential site for therapeutics or design a broad-spectrum vaccine against all strains of HPV. Many proteins, such as transcription factors, bind to DNA sequences within their genome to initiate different biological processes. In the human papillomavirus (HPV), the E2 protein controls the initiation of DNA replication and transcriptional activation through interaction with its binding sites. The four binding sites for the E2 protein are in a conserved spatial arrangement that includes a 12 bp consensus sequence, ACCG(N4)CGGT. This study investigates how the HPV E2 protein and its binding site variants alter binding affinity and kinetics. Binding affinity was initially measured using a fluorescent-based electrophoretic mobility shift assay (EMSA) and further analyzed using Biolayer Interferometry (BLI) to additionally measure binding kinetics. The results determined that known mutations in the binding site consensus sequence altered the binding capacity. The altered binding capacity correlated with the HPV strains that have been classified as high-risk carcinogenic strains.

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Abstract 1775

Ca2+ Induces Dimeric Dual Oxidase's EF-hand Domain to Monomer: an implication for its activation Mechanism

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Amena Razzak, Nickolas McDonald, Hadis Ghasemi

Dual Oxidase (Duox) is a member of the NADPH oxidase (NOX) family that is responsible for the production of H2O2 for thyroid hormone synthesis and innate immune responses. Ca2+ plays a crucial role in the activation of Duox, but little is known about Duox's Ca2+ binding properties. Duox contains two putative EF-hand motifs (EFs), which suggests that its activation mechanism may involve dimerization of its EF-hand domain (EFD). Here, we prepared a truncated Duox EFD (residues 808-902) and used techniques such as fluorescence, calorimetry, electrophoresis, and gel filtration to determine its Ca2+ binding affinity and associated conformational changes. Our results revealed that (1) Ca2+ binding induces conformational changes by exposing hydrophobic patches and burying negatively charged residues, rendering the structure more stable by increasing its melting temperature, (2) Ca2+ binding dissociation constants are determined to be 10 and 0.1 microM for the 1st and the 2nd EF-hands, respectively, in which the bindings appear independent, and (3) apo Duox's EFD forms a dimer and the Ca2+ binding shifts the equilibrium towards the monomer. Using mutagenesis by "knocking" out each Ca2+ binding site in Duox's EFD, we concluded that the 2nd EF is important for dimerization as the Ca2+ knock out in the 1st EF renders a major monomer formation. Since the membrane-bound Duox forms a dimer through inter-domain interactions, we hypothesized that in the absence of Ca2+, EFD serves an inhibitory role by blocking electron transfer while Ca2+ binding mobilizes EFD structure, lifting the inhibition for hydrogen peroxide formation.

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Abstract 1799

Characterizing the specificity of LanCL enzymes for glutathione addition

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Although the formation of dehydrated amino acids is relatively rare in humans, there are both enzymatic and non-enzymatic paths that lead to this type of post-translational modification. Amino acid dehydration leads to protein crosslinking, resulting in protein damage. This occurrence is mitigated by glutathione addition. LanCL1 and LanCL2 enzymes are expressed in various mammalian tissues and were recently discovered to act as catalysts for the addition of glutathione to dehydroalanine (Dha) and dehydobutyrine (Dhb). Exploring the specificity of LanCLs would allow us to understand the scope of this glutathionylation reaction and gain insights into its importance for mammalian cell function. The elimination of a phosphate group on phosphorylated peptides leads to the formation of dehydroamino acids, so we are using a phospho-proteomic approach to determine the specificity of LanCLs. We produced phosphorylated peptide libraries from HEK293T cells, using enrichment with titanium dioxide. These phosphorylated peptide libraries are alkalized with barium hydroxide to create a dehydrated peptide library. After preparing the library, we can react the dehydrated peptides with LanCLs and examine samples with LC-MS/MS, which will determine the specificity by analysis of amino acid patterns in reacted peptides. Results from LanCL-catalyzed reactions will give insight on the promiscuity of the enzymes and the extent to which they modify the human proteome. In addition, LanCLs potentially have a function of enriching dehydro-peptides, which would simplify ongoing attempts to characterize the human dehydro-proteome.

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Abstract 1812

The Interplay of Acetylation and Ubiquitination

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Protein homeostasis is crucial to maintain healthy cells and is predominantly controlled by the ubiquitin proteasome system (UPS) whereby proteins are tagged with ubiquitin, via a cascade of 3 enzymes, resulting in recognition by the proteasome and subsequent degradation. While some proteins are constitutively recognized and degraded by this system, others are marked as substrates for the UPS by post-translational modifications such as phosphorylation. Recently, acetylation of non-histone proteins has emerged as an important mechanism of regulation for the ubiquitin-proteasome system, particularly at the level of E3 ligase substrate recognition. We have applied chemical biology, biochemistry, molecular biology and multi-omics approaches to explore the interplay of acetylation and ubiquitination and the role of acetylation on protein homeostasis. We have identified a subset of proteins with stability regulated at the level of post-translational acetylation. Using proteomics experiments paired with RNA-Seq we are currently expanding our understanding of the breadth of this effect and aim to generate a database of proteins with intracellular levels directly controlled by acetylation. Furthermore, we have characterized the mechanism of acetylation driven protein degradation of key examples featuring a shared acetyl degron motif, including identifying candidate E3 ligases, acetyl transferases and deacetylating enzymes providing unique insights into the into basic biological processes regulating protein stability.

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Abstract 1822

Developing a method for protein editing in mammalian cells

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Post-translational modifications (PTMs) to proteins can have roles in protein function, protein stability and homeostasis, and protein:protein interactions. It is important that we understand the effects of various PTMs, particularly with respect to human health and disease. An ideal way to study PTMs is by site-specifically installing a PTM in a protein of interest in situ in mammalian cells. Unfortunately, many of the currently available methods for manipulating or installing PTMs in mammalian cells lack the necessary specificity, authenticity, and user-friendliness. Methods such as over-expressing or inhibiting the enzymes that install or remove PTMs can cause global changes to the PTM landscape in cells, which does not reflect the highly dynamic and precise regulation governing PTMs. To fill this gap in the field, we have developed a method for in-cell protein editing to install PTMs and other unnatural amino acids. This technology combines protein trans-splicing and genetic code expansion to enable the rapid installation of authentic PTMs and other useful unnatural amino acids, such as click chemistry handles and photo-crosslinkers, into a user-defined site in proteins inside mammalian cells. To demonstrate the utility of this technology, we have installed the unnatural amino acid p-azido-phenylalanine (pAzF), as well as TAMRA and biotin conjugated by click chemistry to pAzF, into calnexin in mammalian cells. This approach has allowed us to validate our protein editing method by immunoblotting, pulldowns, and microscopy. We have shown that our protein editing method is minimally disruptive, rapid, near traceless, and can be easily multiplexed to incorporate a variety of useful labels as well as PTMs.

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Abstract 1826

Accessing isotopically labeled proteins containing genetically encoded phosphoserine for NMR with optimized expression conditions

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Phosphoserine (pSer) sites are primarily located within disordered protein regions, making it difficult to experimentally ascertain their effects on protein structure and function. Therefore, the production of 15N- (and 13C)-labeled proteins with site-specifically encoded pSer for NMR studies is essential to uncover molecular mechanisms of protein regulation by phosphorylation. While genetic code expansion technologies for the translational installation of pSer in *Escherichia coli* are well established and offer a powerful strategy to produce site-specifically phosphorylated proteins, methodologies to adapt them to minimal or isotope-enriched media have not been described. This shortcoming exists because pSer genetic code expansion expression hosts require the genomic ΔserB mutation, which increases pSer bioavailability but also imposes serine auxotrophy, preventing growth in minimal media used for isotopic labeling of recombinant proteins. Here, by testing different media supplements, we restored normal BL21(DE3) ΔserB growth in labeling media but subsequently observed an increase of phosphatase activity and mis-incorporation not typically seen in standard rich media. After rounds of optimization and adaption of a high-density culture protocol, we were able to obtain ≥10 mg/L homogenously labeled, phosphorylated superfolder GFP. To demonstrate the utility of this method, we also produced the intrinsically disordered serine/arginine-rich region of the SARS-CoV-2 Nucleocapsid protein labeled with 15N and pSer at the key site S188 and observed the resulting peak shift due to phosphorylation by 2D and 3D heteronuclear single quantum correlation analyses. We propose this cost-effective methodology will pave the way for more routine access to pSer-enriched proteins for 2D and 3D NMR analyses.

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Abstract 1831

Kinetic and Stability Studies of the *C. elegans* Proteasome

Grant Johnson, *Wabash College*

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The 26S proteasome is a large, multi-subunit ATP-dependent protease complex critical for preserving cellular homeostasis. While the majority of substrates targeted by the 26S proteasome are polyubiquitinated, the 26S proteasome also degrades substrates in a ubiquitin-independent manner. Our lab seeks to develop a novel conditional ubiquitin-independent protein degradation system using de novo designed protein switches in the nematode worm *C. elegans*. To this end, we are interested in better understanding proteasome activity and stability in *C. elegans*. We purified the 26S proteasome from a *C. elegans* strain where a protein in the 19S regulatory subunit (rpt-3) was tagged with a 3xFLAG tag via CRISPR-Cas-9 gene editing. We then assessed the activity of the purified 26S proteasome to degrade the small fluorogenic peptide, Z-GGL-AMC, relative to whole worm lysate from three different *C. elegans* strains. MG132, a known proteasome inhibitor, was used to determine the baseline fluorescence in our assays, which was subtracted to determine the degradation activity (RFU/min/mg) for each condition. Our results show that worm lysates with wild type levels of proteasome degraded the fluorogenic peptide at a slower rate than glp-1 lysate, known to have higher proteasome levels than wild type animals; however, proteasome activity in lysate is short-lived. Furthermore, the purified proteasome remained stable and active for several weeks at 4 °C. The stability of the purified proteasome allows us to streamline our protein degradation assays by having proteasome routinely available, and potentially reducing variation in proteasomal activity between trials as compared to using lysate. The ability to purify the active conformation of the 26S proteasome from *C. elegans* and store it stably for long periods of time has far reaching applications for use in studying protein degradation, such as *in vitro* optimization of a protein switch to control gene product levels. Further, by comparing the purified proteasome to the proteasome in lysate, we may elucidate nuances in *C. elegans*' proteasome function and stability, increasing our knowledge of proteasome function in invertebrates.

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Abstract 1834

Identification of an Unexpected 4-Hydroxyproline (4-Hyp) in a CHO-Expressed IL-2 Mutein

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Interleukin-2 (IL-2) was the first interleukin to be identified and was subsequently isolated and cloned. Its discovery has played a significant contribution to the understanding of T-cell biology. It was also an early cancer immunotherapy and an *E. coli* expressed version of the molecule has been approved to treat metastatic melanoma and metastatic renal caner. However, the protein has a short *in vivo* half-life and toxic side effects when administered at high dose. Moreover, only about 15% of the patients receiving treatment went into remission. Hence, with the development of protein engineering technology, there is considerable interest in the biopharmaceutical industry to develop an improved version of IL-2. The mature protein consists of a single polypeptide chain with 133 amino acid residues. The N-terminus of the wild type protein is APT3SSSTKKT… and Thr3 has been characterized to be O-glycosylated. During the purification of the N-terminal tagged IL-2(T3A) muteins, LC-MS analysis of the products showed the presence of a significant amount of material that is 16 Da higher in molecular mass. Tryptic digestion of the muteins followed by LC-MS/MS analysis showed that the modification was not an oxidation but rather to be due to prolyl hydroxylation at the second residue. To distinguish whether the modification was the 3 or 4 -positional isomer on the pyrrolidine ring, EThcD was employed to generate a diagnostic w ion but the signal was weak and inconclusive. However, automated Edman degradation of the mutein unequivocally demonstrated that the modification was a 4-Hyp isomer. It is interesting to note that when the T3A mutein was expressed at the C-terminal of a monoclonal antibody as a fusion protein, prolyl hydroxylation was not observed.

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ASBMB

Abstract 1835

Analysis of the Functional Motif 5d5 in a Vaccinia Virus System

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Vaccinia virus (VACV) is an Orthopoxvirus and is used as a vector for the variola (smallpox) vaccine. VACV serves as an ideal vaccine vector due to the use of its virally encoded DNA dependent DNA and RNA polymerases and the stimulation of the hosts' humoral and cell-mediated immune response. While VACV vaccines can trigger an immune response, the likelihood of significant side effects is high. To combat this, attenuated, recombinant VACV vaccines have been generated and are safer to use due to the lack of virulence, but the immune response generated is not ideal for long-lasting protection against pathogens. One proposed mechanism to bridge the gap in immune stimulation in recombinant VACV vectors, while retaining the safety profile of the attenuated vaccines, is by using translation enhancing elements (TEEs). TEEs are sequences within the human genome that have experimentally shown to increase protein levels in a VACV system. One specific TEE, hTEE-658, was shown to be particularly active in conjunction with VACV and has been further characterized. In this analysis, hTEE-658 displayed a 5,000-fold increase in luciferase expression over the traditional VACV synthetic late promoter and other TEE sequences. Continued analysis demonstrated hTEE-658 increases gene expression by enhancing both transcription and translation. Furthermore, stepwise deletion analysis identified a 37 nucleotide functional motif within hTEE-658, titled 5d5, which demonstrated a 2-fold increase in enhancement over the full-length hTEE-658 sequence. This current study explored the effects of 3-4 base substitutions throughout the 5d5 sequence to further confirm the importance of certain nucleotides to enhancement activity. The functional effect of various substitutions was determined in HeLa cells transfected with a luciferase reporter plasmid containing the mutated 5d5 sequence of interest, and then infected with VACV. It was determined that some base substitutions influenced transcriptional and translational activity, whereas others did not, suggesting 5d5 has regions of nucleotides that are crucial for functional activity. Additionally, mutations in the predicted TATA-binding protein (TBP) region within 5d5 significantly reduced activity. To further characterize the importance of TBP on 5d5 function, siRNA knockdown was performed to reduce TBP levels within HeLa cells. Following a three-day incubation, the activity 5d5 in these cells was determined using a transfect-infect assay as above. Preliminary results indicate that with reduced TBP levels in HeLa cells, the transcriptional and translational activity of 5d5 also decreases, suggesting a role for TBP in 5d5 function. Understanding the activity of the 5d5 sequence in a VACV system aids in determining how it may function within a recombinant VACV vaccine vector. Furthermore, the results suggest that incorporation of 5d5 into recombinant VACV vaccine vectors could be a potential application to mediate the lack of protein expression. This could allow attenuated vaccines to retain decreased virulence while still expressing the protein of interest at adequate levels for a robust immune response within the host.

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Abstract 1840

Direct interaction between cpTatC N-terminus with precursor mature domain during chloroplast TAT translocation

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Chloroplasts require ~3000 different proteins in order to be fully functional and over 90% of these proteins are encoded by the nucleus and synthesized in the cytosol. Protein routing of the chloroplast is a complex process as they have to across three membranes (chloroplast outer membrane envelop, chloroplast inner membrane envelope, and thylakoid) in order to translocate into the three aqueous suborganeller compartments (intermembrane space, stroma or thylakoid lumen). All nuclear-encoded-chloroplast-targeted proteins are synthesized as higher molecular weight precursor proteins with an amino terminal signal peptide called, transit peptide which are cleavable after the transport of the precursor. Precursors destined for the thylakoid or thylakoid lumen contain a bipartite transit peptide, which promotes import into the chloroplast stroma and subsequent routing to the thylakoid or lumen. There are two major protein transport systems in the thylakoid membrane, chloroplast (cp) secretion (Sec) system and the cp Twin Arginine Transport (TAT) system, which are evolutionarily conserved and also can be found in the plasma membrane of many extant prokaryotes. The cpTAT system transports fully folded proteins by using the proton motive force (PMF) of photosynthesis as the sole source of energy. The cpTAT translocase is composed of three membrane bound proteins: Tha4, Hcf106, and the cpTatC. cpTatC is the largest protein of the system and has six transmembrane domains with the amino and carboxy-terminal tails located on the stromal side of the thylakoid. The cpTatC protein has an amino-terminal extension, typically containing an extra 70–100 amino acids that is lacking in prokaryotic TatC proteins. The N-terminal extension is not necessary for proper targeting and assembly of cpTat in the thylakoid, but its role in transport is unknown. Hence, we hypothesize that the N-terminal extension of cpTatC in the cpTAT system of higher plants plays a role in transport of the precursors. My project aims to determine the function of N-terminal extension of cpTatC in the TAT pathway of higher plants.In order to determine the direct interaction between precursor mature domain and cpTatC protein, disulfide crosslinking assays will be used. Crosslinking between cpTatC and precursor mature domain requires Cys-containing variants of both proteins. The A137C variant of precursor to the 17 kD subunit of the oxygen evolving complex of photosystem II (tOE17A137C) was used as the precursor for all the crosslinking assays. The Cys-containing variants of cpTatC are obtained by site-directed mutagenesis PCR of a Cys-less cpTatC plasmid construct. To test for interactions, intact chloroplasts are isolated and the radiolabeled precursor cpTatC Cys variants were imported into the chloroplast to localize to the thylakoid membrane. After the import assay, thylakoid membranes containing Cys-substituted cpTatC were harvested and be used to follow transport of the tOE17A137C TAT pathway precursor. Cu(II) phenanthroline (CuP) was used as an oxidant to enhance the formation of disulfide bonds. Samples were analyzed by polyacrylamide gel electrophoresis and fluorography. So far, I performed the crosslinking assay with 16 cpTaC variants and 7 cpTatC variants were tested to show interaction with precursor mature domain during the translocation. Amino acid deletions within the cpTatC N-terminal extension were generated to identify to critical region of interaction.

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Abstract 1848

Utilizing Live Cell Imaging in *Drosophila melanogaster* Salivary Glands to Determine if Resveratrol Treatment Activates Heat Shock Factor DNA Binding

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One major stress response pathway is the heat shock response (HSR), which is mediated by the transcription factor, heat shock factor (HSF). The HSR is activated in cells exposed to conditions that induce protein misfolding, such as: high heat, oxidants, and other chemical stresses. Under such stressors, HSF activates expression of the Hsp70 chaperone, which helps cells deal with protein folding stress. However, HSR activation also leads to an increase in reactive oxygen species (ROS), which can damage cellular molecules. To combat this, cells are known to utilize endogenous antioxidants to scavenge free radicals through redox reactions. Therefore, we previously examined the effect of feeding an exogenous antioxidant, resveratrol, on the ability of wildtype Drosophila to withstand heat stress. Treatment with 100 uM and 400 uM resveratrol increased the ability of the flies to withstand heat stress-induced paralysis. We hypothesize that this result occurred because the flies had increased HSF activity due to the resveratrol treatment. To examine this hypothesis, Drosophila larvae expressing HSF-GFP were dissected to obtain salivary glands. These glands contain large polytene chromosomes that allow for visualization of HSF chromosomal binding using confocal microscopy. The most easily visible binding site is an HSF doublet binding at the Hsp70 loci. Salivary glands at room temperature function as a non-heat shock (NHS) control and exhibit no binding of HSF-GFP at the Hsp70 loci. Salivary glands heated to 37C for 10, 20, 40 minutes function as the positive control and exhibit the expected Hsp70 doublet from HSF-GFP binding of the DNA. We are testing variable concentrations (100 uM, 200 uM, and 400 uM) of resveratrol dissolved in 0.5% DMSO to determine if it activates HSF-GFP binding of the DNA in salivary glands under non-heat shock conditions. Our preliminary data indicates resveratrol treatment does not lead to the recruitment of HSF at HSP70 loci in living polytene nuclei. Follow-up experiments are currently underway to examine the levels of Hsp70 protein in cells treated with resveratrol.

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Abstract 1851

Characterization of a novel peptide binding in HUVEC cells using fluorescent polarization approach

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CUL5 acts as the scaffold protein in the E3 ligase complex in the ubiquitin-dependent protein degradation pathway and has been implicated to play a role in cancer pathways. Overexpression of CUL5 in endothelial cells (HUVEC) inhibits proliferation, whereas inhibition of CUL5 expression induces cellular proliferation. In our search to identify proteins that interact with CUL5, we have isolated a novel peptide (LB) that attenuated cell growth in control HUVEC (PX) but not in cells where CUL5 was knocked out using the CRISPR approach (KO). Thus, the aim of this study was to examine if this effect of LB on HUVEC proliferation was dependent on direct CUL5-LB interaction. Our preliminary results, using fluorescent polarization approach, suggest that fluorescent LB binding in KO cells is attenuated when compared to PX cells. Our current work focuses on further characterizing these interactions and how they affect specific signaling pathways that control cellular growth.

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Abstract 1853

Determining the Effect of KmtR Mutants, E101Q, H102Q, and H111Q on the Metal Binding Affinities

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Mycobacterium tuberculosis is the second leading infectious killer after COVID-19. The bacteria utilizes several metal transport systems to help it survive in the host. With an increase in the number of multiresistant, extensively resistant and totally drug-resistant strains, the development of new therapeutic strategies that target other essential pathways in the bacteria is critical. The bacteria contain several metal transport systems which are necessary for its survival. Additionally, the bacteria has two metalloregulators that are associated with nickel and cobalt export, NmtR and KmtR. The focus of this research is on KmtR, which represses the expression of the genes, cdf (which encodes the export protein) and kmtR. The goal of our research is to identify the residues that are responsible for binding the cognate metals, nickel and cobalt, as well as the noncognate metal, zinc, to KmtR. Mutagenesis studies coupled with metal binding experiments will be used to determine how KmtR binds these metals. The E101Q, H102Q, and H111Q mutants, among others, have been made, expressed, and purified in our lab. Data obtained from Isothermal Titration Calorimetry determined that all three mutant proteins bind cobalt with nanomolar affinities and the H111Q mutant KmtR proteins binds cobalt an order of magnitude weaker than the other two mutant proteins.

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Abstract 1859

Purification and Characterization of the Protein KinD that is involved in *Bacillus subtilis* Biofilm Formation

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Biofilms are important structures in agriculture, bacterial infections, and environmental settings. They are communities of bacteria that grow attached to a surface and encapsulated in a self-made matrix. The bacterium *Bacillus subtilis* forms biofilms that are regulated through a biochemical pathway that begins with four histidine kinases: KinA, KinB, KinC, and KinD, and results in the increased transcription of two main operons: epsA-O and tapA-sipW-tasA, that produce the components of the biofilm matrix. It has been demonstrated that KinA and KinB regulate entry into sporulation while KinC and KinD contribute to biofilm formation itself. To better understand the biofilm pathway of *B. subtilis*, a biochemical characterization of the kinase, KinD, was conducted. KinD is an integral membrane protein with an extracellular sensor domain and an intracellular histidine kinase domain. We hypothesize that KinD is an important protein for binding small molecules and interacting with other proteins in the biofilm pathway. To purify KinD, separate His-tagged constructs of the extracellular and intracellular domains were created. After purification with Nickel resin, the protein constructs were characterized using a gel filtration column. The protein yield has been found to be low and the protein appears to have multiple oligomeric states and is prone to precipitation. Ongoing studies are investigating whether small molecules predicted to bind to KinD can stabilize the protein and prevent precipitation. The KinD protein obtained in these experiments can be used in binding studies with other biofilm proteins to further elucidate the mechanism that contributes to biofilm formation.

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Abstract 1869

Disambiguation of Glaucoma-associated Myocilin mutations: Study of trafficking, aggregation, and interactions

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Accurate predictions of pathogenicity for mutations associated with genetic disease are key to the success of precision medicine. This holds true for inherited missense coding mutations in the myocilin gene (MYOC). Missense mutations concentrated within its olfactomedin (OLF) domain comprise the strongest genetic link to primary open angle glaucoma via a toxic gain of function. However, not all mutations in MYOC cause glaucoma, and common variants are expected to be neutral polymorphisms. The gnomAD database lists ~100 missense variants documented in the within OLF, all of which are relatively rare (allele frequency <0.001%) and nearly none has data on their likelihood of glaucoma pathogenicity. To disambiguate disease from benign OLF variants, we first characterized the most prevalent population-based variants using a tailored cellular fractionation assay. This assay identified three of 17 new variants with features of aggregation-prone familial disease variants. While useful, the lack of quantitation in the assay and low throughput are undesirable. Therefore, we sought alternative methods including modified cellular assays, confocal microscopy, and flow cytometry. Taken together, these efforts increase efficiency with which it is possible to disambiguate disease variants from those likely to be benign with higher throughput. In the long term, these studies support genetic screening of individuals for early monitoring for glaucoma.

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Abstract 1881

The Mammalian Rhomboid-like Derlins Regulate Endoplasmic Reticulum Morphology

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The endoplasmic reticulum (ER) is an essential organelle that is a large hub for protein folding and modification, and largely interconnected with these processes is the protein quality control pathway, Endoplasmic Reticulum Associated Degradation (ERAD). ERAD is important for the degradation of misfolded proteins in the ER and a key protein family important in this process are the rhomboid pseudoproteases, the Derlins. They have largely been characterized in yeast as involved in the retrotranslocation of misfolded membrane proteins by binding Cdc48 (p97 in mammals). We have found that the mammalian Derlin proteins play an important role in the morphology and structure of the ER. Microscopy of HEK293T cells lacking all Derlin proteins (DERL1-3) show a disrupted ER morphology. We found that this morphology is likely connected to the change in stability of the ER structural proteins, the Atlastins (ATL1-3). To survey for potential substrates of derlins, we employed liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomics in derlin knockout (KO) HEK293 cells. To our surprise, ATL-3 was significantly enriched in the derlin KOs, indicating that ATL-3 is a potential substrate of derlins. To better understand these two changes, we further characterized the Atlastin proteins as Derlin substrates. We found that Atlastin protein abundance decreases in DERL1 null mutants as well as the triple null mutant of the Derlin proteins. By understanding how the Derlins regulate the structure and stability of the Atlastin proteins, we can better understand how components of ERAD control ER morphology and how membrane proteins are controlled by the Derlin proteins in the ER. As mutations in the Atlastins are associated with hereditary spastic paraplegia, this study will aid in our understandings of how the Derlins contribute to this disease and others like it.

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Abstract 1887

Using circular dichroism to probe small molecule binding to Peptidoglycan Associated Lipoprotein

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Peptidoglycan-associated lipoprotein (Pal) is a protein whose lipid moiety anchors into the outer membrane of Gram-negative bacteria, such as *Escherichia coli*. Pal has been shown to play important roles in cell division and to contribute to the stability of the cell via its interactions with peptidoglycan and other proteins in the Pal-Tol complex. Pal binds to peptidoglycan through strong non-covalent interactions and to TolB using a similar binding site. We sought to identify small molecules that could interact with Pal at this binding "hot spot." Several small molecule candidates were proposed by participants from the Molecular Interactions Virtual summer research program, funded by the NSF, who used the computational program Dock6 and the ZINC database. Here, we describe results from our circular dichroism analysis of recombinant Pal protein incubated with several small molecule candidates, including FMN. Preliminary results show that FMN changes the thermal denaturation curve of Pal, suggesting that FMN does interact with Pal. Further structural analysis is underway to identify the small molecule binding site(s), with the long-term goal of evaluating the small molecules for their effect on the Pal-TolB interaction, as well as Pal's role in cell division.

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Abstract 1898

Structure of the *E. coli* DNA repair protein RarA

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The *E. coli* RarA protein is part of a family including one of the most conserved set of proteins from bacteria to humans. Homologs include Mgs1 (yeast) and WRNIP1 (humans). RarA is a AAA+ ATPase and also homologous to the DnaX clamp loader but functions as a tetramer. RarA is clearly involved in some aspect of DNA metabolism, implicated in roles in the RecFOR pathway of postreplication gap repair and in RecA-independent intermolecular recombination. However, little is known about the detailed molecular function of this protein or any of its family members. The RarA protein structure has been determined in its apo form. To gain further insight into RarA structure and function, we have initiated efforts to solve the structure of additional protein forms, including those bound to ATP and/or DNA, using Cryo-electron microscopy. In this poster, we will present the initial challenges of analyzing the functional form of RarA and its mutants under Cryo-EM and how combining the results from negative stain helps decoding the structures. No human subjects or animal data are used in this study.

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Abstract 1899

Effect of Aβ42 on Alzheimer's development

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Alzheimer's is a dementia disease caused by a corrupted form of amyloid-beta precursor protein that destroys nerve cells in the brain. For a long time, it has been known that Alzheimer's was caused by the buildup of amyloid plaques in the brain. However, these plaques are formed by decreasing levels of amyloid-beta in the brain. Amyloid-beta is decreased because the normal version of the protein under proper circumstances transforms into the abnormal amyloid plaques. There is a direct correlation (among people who are genetically at risk of developing Alzheimer's) between the presence of high levels of a specific form of amyloid, called soluble Aβ42, and the reduced risk of memory loss. It is a group interest to study the formation of soluble Aβ42 and its function in order to possibly find the reason why the high amounts of this protein reduce the risk of having Alzheimer's. The research will also include an analysis of the structural differences between Aβ42 and Aβ40. Brain proteins function when their soluble/native conformation is transformed into amyloids (which are the insoluble aggregates). β- and γ-secretases are amyloid precursor proteins that produce Aβ, which is a proteolytic product. There exists a cleavage of γ-secretase at C-terminus that produces the two most important Aβ isoforms: Aβ42 (42 residues long) and Aβ40 (40 residues long). The main structural difference in both lies in the two additional C-terminal residues on Aβ42.

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Abstract 1924

A Comparative Analysis of Translation Enhancing Element Activity

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Protein production involves a complex series of intracellular events which can be broken down into the two major processes of transcription and translation. Translation occurs in three phases: initiation, elongation, and termination. During the initiation phase, the ribosomal subunits and translation machinery are recruited to the messenger RNA (mRNA) produced during transcription. This recruitment can occur via cap-dependent or cap-independent mechanisms. Cap-dependent translation initiation occurs when the 5′ m7G cap —present on the mRNA—is recognized by the eIF4F complex, whereas cap-independent initiation occurs when translation machinery is recruited to the mRNA without the presence or recognition of the 5′ cap. A growing body of evidence has shown that eukaryotic cap-independent translation initiation can occur under conditions of cellular stress, such as during mitosis and apoptosis, and during viral infections. However, much is uncertain regarding the exact mechanisms of cellular cap-independent translation initiation and the sequences capable of stimulating this non-traditional mechanism of protein production. To address this, previous research identified Translation Enhancing Elements (TEEs), which are sequences found throughout the human genome that have the potential to facilitate cap-independent translation initiation. In this research, a subset of the TEEs were functionally characterized using a transfect-infect model with the vaccinia virus to assess their protein production capabilities. This current study aims to further characterize nine of these sequences which had greatly increased protein production activity above that of a random genomic control sequence. The selected sequences underwent both functional and structural analyses to assess protein production capabilities and potential functionality within the genome. Structural analysis revealed the precise genomic location of each sequence and a predicted lack of secondary structure formation—consistent with other identified mechanisms of cellular cap-independent translation initiation. Functional analysis employed a reporter construct that contained the TEE sequence of interest upstream of the firefly luciferase gene, which was then used to produce mRNA *in vitro* for transfection into HeLa cells. The effect of the TEE was then determined using luciferase assays and quantitative real-time PCR to assess protein production activity levels relative to a random genomic control segment. When assayed under these conditions, the sequences displayed a range of protein production activity. Under cap-independent conditions, the sequences also displayed varying effects on RNA stability across time points. Overall, understanding the structure and function of these TEE sequences could yield important information regarding the translation of proteins from the human genome,

along with furthering our knowledge of cellular translation initiation. Additionally, understanding the activity of these sequences could yield promising potential for increasing the effectiveness of RNA-based technologies.

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Abstract 1926

Investigating the Role of Arginine Methylation in PGC-1α, a Temperature Dependent Metabolic Regulator

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The protein peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α; UniProt Q9UBK2) plays an important role in the regulation of energy metabolism and is implicated in type two diabetes. To modulate its activity, PGC-1α becomes methylated at many arginine residues, however the location and significance of this modification is unclear. Moreover, since arginine methylation results in the addition of one or two methyl groups, the regulatory role of these distinct groups and functional outcomes for PGC-1α remain to be seen. Using fluorography and mass spectrometry we have determined that the C-terminus (amino acids 481–798) of PGC-1α, is an arginine methyltransferase substrate of the methyltransferase enzyme PRMT7. We performed *in vitro* methylation reactions using recombinant mammalian PRMT7 at temperatures ranging from 4°C to 37°C and found that arginine residues R548 and R753 in PGC-1α are methylated at or below 30°C by PRMT7. We also employed prediction programs such as PRmePRed, and MePred-RF to search for putative methyltransferase sites. Computational approaches yielded additional putative methylarginine sites, indicating that additional methylated arginine residues have yet to be experimentally verified. To understand the physiological consequence of these modifications, *in vivo* experiments conducted at varying temperatures are underway. We conclude that PRMT7 methylates PGC-1α, with more methylation occurring below physiological temperature, uncovering an additional control point for PGC-1α.

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Abstract 1952

Quantification of Whey Proteins in Bovine Milk Samples: A Differential Scanning Calorimeter Approach

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Bovine milk is composed of various proteins which aid in different human bodily functions. β-Lactoglobulin (β-Lg), α-Lactalbumin (α-La), bovine serum albumin (BSA), lactoperoxidase (LP), and lactoferrin (LF) are all found within whey which is produced on a large-scale during cheese production. β-Lg, the most prevalent protein in whey, can be an allergen so it is important to know the concentration of β-Lg in a given sample before selling dairy products. α-La plays a key role in lactose production and inhibiting bacterial growth. BSA is an anticoagulant and antioxidant; BSA is also used in many different scientific experiments as a standard and a reagent. LP is also an antioxidant and has antibacterial properties. Lastly, LF, an iron binding protein found in milk whey, has antibacterial, antifungal, and antiviral properties. Concentrations of these 5 different proteins can vary widely between whey and milk samples; often, purification of these proteins requires knowing their concentration in each sample. Currently, protein concentration is determined through ELIZAs which contain many steps and can take up to 10 hours. There is a significant need to quantify whey proteins in a rapid manner in order to decrease purification time of these biologically active proteins. We propose that whey proteins from a bovine milk sample can be quantified by Differential Scanning Calorimetry (DSC). DSC measures the temperature of unfolding (Tmax) and the enthalpy of unfolding (ΔH); it has been used to characterize cancer protein profiles, measure chemical reactions, and study oxidation. In large, the DSC has been used for qualitative measures. However, it has never been used quantitatively to determine concentrations of proteins. We demonstrate that the DSC gives a certain denaturation profile for each milk protein and that these profiles can be used to quantify the milk proteins. We have first demonstrated the quantification of lactoferrin using the DSC. Specifically, the lactoferrin denaturation profile has 2 peaks; a lower peak approximately 61°C where the apo form of lactoferrin denatures and a peak around 87°C where the holo form of lactoferrin denatures. By inducing lactoferrin into the holo form in a sample, we have separated lactoferrin from other whey proteins and use the area under the curve and lactoferrin's ΔH, determined by the DSC, to calculate the concentration of lactoferrin in a given sample. We have found that the DSC can detect a difference of a 1 mg/ml addition of protein to a sample. Decreasing quantification and purification times of whey proteins will have a large effect on dairy companies enabling them to isolate whey proteins much quicker. This will allow faster research on beneficial health properties of whey proteins and increase the number of products produced requiring the addition of whey proteins.

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Abstract 1961

Creation of dimeric *Perkinsus marinus* creatine kinase through site-directed mutagenesis: Insight into phosphagen kinase quaternary structure evolution

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Cytosolic creatine kinases (CK) play a central role in buffering ATP levels and are obligate dimers; a single subunit cannot function without the presence of the other. However, a recent discovery revealed that novel CKs in *Perkinsus marinus* (PmCK), a parasitic protozoan, comprise only a single subunit yet remain completely active. Amino acid sequence alignment indicated that PmCKs are missing some key residues involved in dimerization as identified in dimeric CKs; hence they exist as monomers. However, the monomeric state of PmCK is evolutionarily in query: whether it lost the ability to dimerize or is only a few mutations away from becoming a dimer. To understand how the change in key residues affects the quaternary structure of PmCK, site-directed mutagenesis will be used to introduce key residues (D54G, F178R, and G179D) involved in dimerization in dimeric CKs into PmCK. Size-exclusion chromatography and native gel will test whether mutants with more than two key residues are dimers. Linked-enzyme assay will show that catalytic abilities may or may not be retained since the key residues might interfere with the active sites of the mutants. The results from these studies will help us better understand how dimerization might have arose in the CK family.

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Abstract 1962

Structure and mechanism of Neurexin - Adhesion GPCR interactions in neuronal Synapses

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Julia Brasch

Synaptic adhesion molecules (SAMs) are found ubiquitously in neuronal synapses that keep terminals of neurons in contact for neurotransmission and to establish neural circuits. Mutations in SAMs are linked to neurological disorders like autism, due to dysfunction in synapse formation and neurotransmission. Understanding the structures and interactions of SAMs on molecular detail is essential to understanding proper synapse function and synaptic disorders. Neurexins (Nrxs) are critical SAMs that are thought to organize the extracellular space of synapses through interactions to at least eleven other SAMs. Most of these interactions are crucial to promote synapse formation. A pan-neurexin deletion is lethal in mice, while deletions of individual members of the neurexin family cause a reduction in synaptic transmission. Adhesion GPCRs (aGPCRs) are another crucial group of SAMs. aGPCRs are important in cells as they sense changes in the cell's exterior environment and communicate these to the cell, making aGPCRs excellent drug targets. ADGRB3 (also known as Brain angiogenesis inhibitor 3) is a postsynaptic aGPCR that regulates synaptogenesis, synapse refinement, and synapse elimination. Both Nrxs and ADGRB3 play roles in synapse formation, yet no information regarding the possible interaction of these two proteins is known. Interestingly, previous studies show that Nrxs and ADGRB3 each can interact with soluble synaptic adhesion molecules, C1qls. We propose that C1qls bridge presynaptic Nrxs and postsynaptic ADGRB3s forming large heterocomplexes essential for their synaptic roles. This study aims to determine the molecular basis of this interaction using a combined biophysical, biochemical, and structural biology approach. To study the putative Nrx-C1ql-ADGRB3 interaction, we have produced full-length histidine-tagged extracellular domains of Nrx, C1qls, and ADGRB3. First, through biophysical experiments using multiangle light scattering, we show for the first time that ADGRB3 binds to distinct oligomeric states of C1qls, likely creating more interaction regions for binding to other ligands. Secondly, using bead-based binding experiments, we have identified a novel synaptic supercomplex formed between Nrxs, C1qls, and ADGRB3, confirming our prediction. Structural studies of these complex are underway to determine the molecular details of this interaction. We also aim to understand how the presynaptic ADGRB3 ligand, Nrx, activates ADGRB3 to promote aGPCR signaling and synapse formation. Overall, this project will explain how important components of the synaptic cleft interact to promote proper synapse function and further provide novel insight into how mutations in Nrxs, C1qls, and ADGRB3 may affect synapse formation and

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neurotransmission in disease, which is the basis of common neurological disorders.

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Abstract 1967

Biophysical characterization of the BqsR response regulator from the *Pseudomonas aeruginosa* BqsR/S two-component system

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Pseudomonas aeruginosa (Pa), a ubiquitous Gram-negative bacterium best known for infecting the lungs of cystic fibrosis (CF) patients, is one of the major causes of chronic nosocomial infections and can grow either as planktonic or biofilm. Biofilms are complex microbial structures capable of providing an advantageous protective quality that causes bacteria living within a biofilm to be significantly more resistant to antibiotic treatments than planktonic bacteria, representing a major health threat. Recent studies have uncovered a novel two-component signal transduction system that regulates biofilm formation/decay in *P. aeruginosa* through extracellular Fe2+ binding known as BqsR/S. The sensing of Fe2+ is important, as this nutrient is present throughout each stage of infection in CF sputum and constitutes a large portion of the iron pool present in advanced stages of lung function failure. PaBqsS has been identified as a transmembrane sensor kinase while PaBqsR has been identified as a cytosolic response regulator that binds to DNA and is capable of altering transcription of genes involved in biofilm formation. However, neither of these proteins have been structurally characterized, and the details of how and to what extent they interact with Fe2+ remain unknown. In this work, we have expressed, purified, and initially characterized PaBqsR using NMR structural techniques. These results reveal the presence of a well-folded, monomeric protein in the absence of phosphorylation. Using X-ray crystallography, we have solved the structure of the N-terminal phosphorylation domain of PaBqsR to 1.3 Å resolution, revealing a canonical (βα)5 response regulator assembly that consists of a central five-stranded parallel β-sheet surrounded by five helices. Surprisingly, generation of two phosphorylation mimics (a D51E PaBqsR variant and a BeF3−incubated form of PaBqsR) maintains the monomeric oligomerization of the response regulator based on gel filtration studies. Interestingly, we have found that the reported PaBqsR DNA-binding consensus sequence is located upstream of the feo operon, the primary Fe2+ transporter of *P. aeruginosa*. Preliminary electrophoretic mobility shift assays (EMSAs) show that PaBqsR is indeed capable of binding upstream of the feo operon, but only in the pseudo phosphorylated state. These results provide the first biophysical characterization of the Bqs system and demonstrate an unexpected connection between the BqsR/S system and the Feo system, an important *P. aeruginosa* virulence factor.

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Abstract 1974

Production of Tandem Repeat Reflectin Proteins with Tunable Properties Using Rolling Circle Amplification

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Reflectin is an intrinsically disordered block copolymer protein located in cephalopod iridocytes, which are reflective skin cells composed of invaginated reflectin-filled nanostructures. Phosphorylation of reflectin induces tunable iridescence, which plays a role in cephalopod communication and camouflage. The exact relationship between reflectin's unique repetitive amino acid sequence and composition and its biophysical or physiological properties remains elusive. This work focuses on developing a method of producing deviation-free tandem repeat reflectin proteins for the scalable investigation of the role of reflectin sequence features on reflectin assembly, dynamic arrest, and the material properties of reflectin condensates. To produce our desired tandem repeat reflectins, we adapted a molecular cloning method called PD-RCA (Protected Digestion of Rolling-Circle Amplicons). Using this method, we were able to produce short reflectin constructs. Optimizations to the PD-RCA method resulted in accelerated amplification times and improved tandem repeat DNA fragments yields, though the yield of highly mutated constructs was also high. We continue to evaluate and optimize this method for the future higher-throughput production of a range of tandem repeat proteins.

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Abstract 1984

Characterization of 53BP1-LC8 binding in the presence and absence of the oligomerization domain

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The tumor-suppressor p53 binding protein 1 (53BP1) plays a critical role in the DNA damage response and the regulation of the cell cycle. 53BP1 has an oligomerization domain (OD) and a dynein light chain 8 (LC8) binding domain (LBD), both of which are important for DNA damage repair function. LC8 is a dimeric hub protein which is known to dimerize over 100 intrinsically disordered client proteins. The interactions of LC8 with client proteins are often multivalent, and previous work has shown the existence of three LC8 binding sites within the LC8 binding domain of 53BP1. Isothermal titration calorimetry on single-site and double-site 53BP1 mutants was used to characterize LC8 binding in the presence and absence of the oligomerization domain. Interestingly, while the oligomerization domain does not drastically affect the thermodynamics or affinity of the binding interaction, it does affect the binding mechanism. SEC-MALS of the 53BP1 LBD and the 53BP1 LBD-OD reveals the formation of larger-order complexes in the presence of the oligomerization domain, hinting at a new binding mode for LC8.

This work is funded by the National Institutes of Health (R01GM141733 to EB). We also acknowledge the support of the Oregon State University NMR Facility funded by the National Institutes of Health, HEI Grant 1S10OD018518, and the M. J. Murdock Charitable Trust grant #2014162.

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ASBMB

Abstract 1987

DNA dependent regulation of the Lon protease

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Regulated protein degradation is essential for protein homeostasis but must be tightly controlled as it is an irreversible process. AAA+ proteases like Lon are responsible for most of protein degradation in the cell. In eukaryotes, deletion of Lon is embryonic lethal and defects in mitochondrial Lon result in defects in mitochondrial DNA maintenance and in mitochondrial stress adaptation. In bacteria loss of Lon results in growth defects, lowered motility, and a diminished stress response. While Lon is known to bind DNA, it is not known how DNA binding affects Lon activity. Here we find that Lon's DNA binding can affect both proteolytic function and ATP hydrolysis activity depending on sequence and topology. Double stranded DNA acts as a scaffold to recruit Lon to DNA, where it clears DNA-bound proteins during genotoxic stress. Bacterial Lon binds single-stranded DNA more tightly than double-stranded DNA and this interaction results in an increase in ATP hydrolysis, changes in proteolytic activation and nucleotide affinity. These effects are sequence dependent. DNA binding data and mutational analysis of the putative DNA binding domain suggests Lon can adopt a more active state. Together these data inform on a model of Lon adopting various activation states to regulate proteolysis.

104092, https://doi.org/10.1016/j.jbc.2023.104092

Abstract 1994

A new method for quantification of tRNA aminoacetylation levels using sequencing

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The aminoacetylation level of tRNAs is a fundamental variable in cell biology which is difficult to measure. Recently, it was shown that tRNAs with an aminoacylation are protected against periodate oxidation of the 3′ ribose and subsequent cleavage of the oxidized terminal nucleobase. Thus, making it possible to discriminate between tRNAs with/without aminoacylation using sequencing. We wanted to combine the recent developments in tRNA sequencing methods, with old observations on the chemistry of the terminal nucleobase cleavage, to make a new method to quantify tRNA aminoacylation levels. We found several improvements: 1) decreased time/temperature during periodate oxidation to prevent hydrolysis of the labile aminoacylation, 2) amine-induced cleavage at lower pH to preserve RNA integrity, 3) combination of oxidation/cleavage in a one-pot reaction, 4) a splint-assisted ligation method to achieve ∼100% adapter ligation thus making this step quantitative. In summary, we improved the reaction central to tRNA aminoacylation discrimination and found that quantitative aminoacylation levels could be obtained using splint-based ligation. Furthermore, we designed and tested a set of barcoded adapters and primers for two layers of multiplexing, a spike-in control of the cleavage reaction and a pipeline for data analysis. With this, we hope to increase the availability and quality of tRNA aminoacetylation level measurements.

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Abstract 1997

Assembling a structural model of the SARS-CoV-2 replication and transcription complex from 60+ subunits

Jason Perry, *Gilead Sciences*

The SARS-CoV-2 replication and transcription complex (RTC) is made up of nine distinct non-structural viral proteins encoded by the ORF1ab gene. These proteins house seven enzymatic sites that synthesize new viral genomic and subgenomic RNA, proofread and correct errors in the synthesis, add a 5′-cap to the nascent RNA, and truncate the intermediate negative sense 5′-poly-U tail. While x-ray crystallography and cryo-EM have provided high resolution structures of each of the individual proteins of the RTC and have shed light on how subsets of the proteins associate, a full picture of the RTC has remained elusive. Using molecular modeling tools, including protein-protein docking, we have generated a model of the RTC centered around hexameric nsp15, which is capped on two faces by trimers of nsp14/nsp16/(nsp10)2. A conformational change of nsp14, necessary to facilitate binding to nsp15, then recruits six nsp12/nsp7/(nsp8)2 polymerase subunits. To this, six nsp13 subunits are distributed around the complex. The resulting superstructure is composed of 60 subunits total and positions the nsp14 exonuclease and nsp15 endonuclease sites in line with the dsRNA exiting the nsp12 polymerase site. Nsp10 acts to separate the RNA strands, directing the nascent strand to the nsp12 NiRAN site, where a transiently associated nsp9 facilitates the first step in mRNA capping. The RNA is then directed to the nsp14 N7-methyltransferase site and the nsp16 2′O-methyltransferase site to complete the capping. Additionally, template switching during transcription is proposed to be facilitated by positioning of the TRS-L RNA-bound N-protein above the polymerase active site, between two subunits of nsp13. The model, while constructed based on structural considerations, offers a unifying set of hypotheses to explain the diverse set of processes involved in coronavirus genome replication and transcription.

All work presented was funded by Gilead Sciences.

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Abstract 1999

Structural and Functional Analysis of Cavia porcellus and Human Apolipoprotein E4 to Understand its Role in Amyloidogenesis

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Amyloid-β (Aβ) peptide aggregation and senile plaque formation are hallmark features of Alzheimer's disease (AD), a neurodegenerative disease characterized by cognitive decline. One of the risk factors for AD is the inheritance of the APOE e4 allele that encodes the protein apolipoprotein E4 (apoE4). Several studies have demonstrated the presence of apoE4 in the amyloid plaques, prompting the question of the role of apoE4 in amyloid plaque formation or amyloidogenesis. Other researchers have suggested that the C-terminal domain of apoE4 plays a role in interaction with lipids, Aβ peptide and other apoE4 molecules to form a tetramer. The current study seeks to understand the structure and function of apoE4 and its role in amyloid formation. Sequence alignment of human apoE4 with apoE from Cavia porcellus, guinea pig (GP) shows that the latter is only 281 aa long while the former is 299 aa long. A major difference is that GP apoE has two deletions corresponding to residues 193–197 and 246–252 (in the C-terminal domain) in apoE4. GP apoE was used to understand the role of these residues in apoE4. Recombinant GP apoE was overexpressed, isolated, and purified in *E. coli*. Biophysical studies were carried out with GP apoE and apoE4 to gain understanding of the tertiary fold around these segments of apoE4. 1-anilinonaphthalene-8-sulfonic acid (ANS)fluorescence emission spectra presented a blue shift in GP apoE in relation to apoE4 and apoE3 (a polymorph of apoE4 that is present in a majority of the population), indicating an increased level of protein surface hydrophobicity. We are currently performing trypsin digestion of each wild type apoE to compare resistance to proteolytic degradation. Taken together, understanding structural and functional behavior of GP apoE may offer insights into the mechanistic basis of apoE4's role in AD.

This work was supported by the National Institutes of Health (grant# NIH-GM105561 (VN)), NSF LSAMP Program fellowship (HRD-182649) (JN) and NIH RISE grant (T32GM138075) (GC).

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Abstract 2003

Pursuing Fundamental Mechanisms of Biofilm Formation in *Acinetobacter baumannii*

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Acinetobacter baumannii is a pathogen that can form biofilms – multicellular communities of bacteria within a self-made environment of various proteins, sugars, and nucleic acids. These biofilms protect the bacteria from stressors such as antibiotics and desiccation, which causes difficulty in treatment and prevention of illnesses caused by this pathogen. It has recently been shown that Lon protease promotes biofilm formation in *A. baumannii*. Additionally, Lon transcriptionally downregulates SurA1, a surface protein involved in biofilm formation and virulence. In accordance with Lon's ability to bind to DNA in other species of bacteria, we believe that Lon is affecting SurA1's expression levels by binding to DNA in *A. baumannii*, either by degrading a transcriptional activator or directly acting as a repressor. To determine the relationship between Lon and SurA1, we used an assay that combines key features of CRISPRi and ChIP-seq procedures. By programming a CRISPRi system to cause transcriptional interference on the surA1 ORF, we were able to position Cas9 to immunoprecipitate the complex. This would allow us to pull down any proteins bound to the surA1 promoter region and identify them via mass spectrometry. By identifying the proteins controlling SurA1 expression, we can better define the relationship between this protein and Lon, both of which influence the formation of biofilms in *A. baumannii*. With this knowledge, we will be better equipped to design methods to prevent the spread of this bacterium, and we have refined a technique that can be used to identify bacterial transcriptional regulators.

This research was funded by three 'Project-Based Exploration for the Advancement of Knowledge' (PEAK) Experiences Awards from Northeastern University's Office for Undergraduate Research and Fellowships.

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Abstract 2004

Zinc binding to the N-terminal domain of postsynaptic density protein 95 impairs its palmitoyl modification

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Xiaoqian Fang

Postsynaptic density protein-95 (PSD-95) is a primary postsynaptic membrane-associated protein and the major scaffolding component in the excitatory postsynaptic densities, which performs substantial functions in synaptic development and maturation. PSD-95 mediates postsynaptic location of AMPA receptors through its N-terminal C3/C5 palmitoylation/depalmitoylation switch. Its membrane association induced by palmitoylation contributes largely to its regulatory functions at postsynaptic sites. Amino acid sequence analysis revealed PSD-95 N-terminus consists of a putative zinc-binding motif with undiscovered functions. This study was to investigate zinc-binding to the N-terminal domain and its effect on PSD-95 palmitoyl modification. The NMR titration of 15N-labeled PSD-95NT by ZnCl2 was performed and demonstrated Zn2+ binds to PSD-95NT with a binding affinity (Kd) in the micromolar range. The zinc binding was confirmed by fluorescence and mutagenesis assays, indicating two cysteines and two histidines (H24, H28) are critical residues for the binding. These results suggested the concentration-dependent zinc binding is likely to influence PSD-95 palmitoylation since the binding site overlaps the palmitoylation sites, which was verified by the mimic PSD-95 palmitoyl modification and intact cell palmitoylation assays. This study concluded that zinc binding to the N-terminal domain of PSD-95 negatively regulates its palmitoyl modification, indicative of the importance in postsynaptic signaling.

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Abstract 2022

Regulation of Estrogen Receptor Signaling by FKBP52 in Breast Cancer Cells

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Marc Cox, Rina Koyani, Anapaula Camou

Estrogen receptors (ER) are nuclear transcription factors that are regulated by estrogens and have been considered responsible for enhancing proliferation in breast cancer. The FK506-binding protein, known as FKBP52, has been shown to associate with steroid receptor complexes *in vitro* and is suggested to act as a positive regulator of ER expression in some breast cancers. However, the physiological role of FKBP52 in ER-mediated cell function has not been extensively studied. Our strategy involved the usage of the CRISPR/Cas9 gene-editing system to create fkbp52-deficient (52KO) lineages of T-47D and MCF-7 breast cancer cells to assess the impact of FKBP52 loss. We were able to verify our 52KO lineages through western blot analysis. Using MTT cell proliferation assays, we found that FKBP52 depletion resulted in decreased cell proliferation when compared to both wild-type (WT) T-47D and MCF-7 cells. Subsequently, we analyzed cellular migration and visualized the inhibition of migration in fkbp52-deficient cell lines. Additionally, reporter assays were also performed to examine the effect that estradiol might have on ER transcriptional activity in both WT and KO cell lines. Our findings show that suppression of FKBP52 has influence on the cellular proliferation and migration of ER-positive breast cancer cell lines. This study could generate useful data on a target for the exploitation of the ER-signaling pathway in the treatment of breast cancer.

This research was funded through the U-RISE program at the University of Texas at El Paso (UTEP). U-RISE is a program that provides stipends to undergraduate students in research and it is a National Institute of General Medical Sciences (NIGMS) funded program.

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Abstract 2023

Fluorescence Spectroscopic Analysis of Apolipoprotein AI Reconstituted High Density Lipoprotein

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High density lipoproteins (HDL) are protein-lipid complexes that aid in cholesterol efflux, a process in which HDL particles interact with ATP-binding cassette transporters ABCA1 and ABCG1 to remove excess cholesterol from macrophages. The major protein on HDL, apolipoprotein AI (apoAI), exists in lipid-free and lipid-bound states. When bound to phospholipids, it contains 10 a-helices (H1-H10) that wrap around the hydrophobic lipid tails. Our goal is to understand how the conformation of apoAI changes during cholesterol efflux. We hypothesize that residues 125–158 (helices H5 and H6) form a disordered loop that can accommodate lipid loading and changes in HDL particle size. To test this hypothesis, an apoAI double-cysteine mutant (L134C/A152C) was designed with cysteines positioned on the loop. The purified protein was labeled with N-(1-pyrene)-maleimide (NPM), a spatially sensitive fluorophore that has a distinct emission at ~460 nm when it is within ~10 Å of a neighboring pyrene. The pyrene-labeled apoAI was reconstituted with phospholipids at different phospholipid:protein molar ratios (28:1, 70:1, and 100:1) to generate small (~7.8 nm), medium (~9.6 nm) and large (~10.5 nm) diameter HDL referred to as reconstituted HDL (rHDL). The pyrene-labeled rHDL was incubated with J774.1 macrophages undergoing cholesterol efflux, and fluorescence spectra compared before and after efflux. In the rHDL-bound state, apoAI undergoes conformational reorganization with a significant decrease in excimer emission. The data suggests that the conformational changes accommodate for lipid loading, but more studies are required to obtain further details about the conformational reorganization during lipid loading of HDL.

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Abstract 2024

Employing Solution NMR to Study BTK Activation Through Sortase-Mediated Ligation and Paramagnetic Relaxation Enhancement

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Bruton's Tyrosine Kinase (BTK) is a member of the Tec family non-receptor kinases and is an essential component of the B-cell signaling cascade, contributing to both B-cell development and antibody production. BTK is of clinical importance as it is the target of Ibrutinib, which is used for treatment of chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). Mutations in the BTK gene also cause a genetic immunodeficiency disorder, known as X-Linked Agammaglobulinemia (XLA). There are numerous XLA mutations identified throughout the BTK enzyme, but our attention focuses on a cluster of mutations residing in the BTK SH2 domain that we hypothesize is part of a regulatory interface that positively influences kinase activity. To investigate this further, I employed sortase-mediated ligation (SML) to generate a segmentally isotopic labeled protein that was analyzed using solution nuclear magnetic resonance (NMR) spectroscopy. The partially labeled protein allowed us to identify an intradomain regulatory interaction between the BTK SH2 and catalytic kinase domain. Further, the BTK SH2-Kinase protein was studied using an NMR technique known as paramagnetic relaxation enhancement (PRE) which informs on surface exposed amino acid residues, aiding the identification of a potential intramolecular interface. From the NMR data, an activation-state model of BTK has been constructed using computational modeling and I am currently testing this model using mutagenesis and coupled-kinase activity assays. The results provided include NMR spectra from the SML and PRE experiments, an activation state model of BTK, and preliminary data from the activity assays. We anticipate that interrogation of the SH2-kinase interface will elucidate the molecular mechanisms that govern BTK activation, a phenomenon that is less well understood for Tec family kinases. These efforts contribute to the development of drug therapies for modulating kinase activity within the immune cell.

This work is supported by the NIH/NIAID Structural Studies of a T cell Specific Tyrosine Kinase grant 5 R01 AI043957-24, the Roy J. Carver Charitable Trust and the Iowa State University Graduate Minority Assistantship Program (GMAP).

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Abstract 2025

The Role of Calcium and Zinc in the Amyloid Formation of Insulin

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Khevana Patel, Marianna Torok

Amyloids are highly insoluble protein aggregates with fibrillar morphology and common physicochemical properties. The accumulation of toxic amyloids often leads to cell death or failure to function adequately, thus they are associated with many human diseases including Alzheimer's disease, type II diabetes, and cancer. Insulin is known to form amyloid balls at injection sites which can trigger loss of glycemic control. Additionally, *in vivo* studies have shown that insulin amyloids have a cytotoxic effect and can strongly interact with erythrocytes indicating that the effect of insulin amyloidogenesis may be greater than a therapeutic problem. Calcium and zinc both contribute to the stability of insulin, however metal cation dyshomeostasis has been linked to a variety of diseases including type II diabetes. In this study, the effects of the Ca2+ and Zn2+ concentrations on insulin aggregation kinetics and morphology are investigated under acidic conditions using thioflavin T fluorescence spectroscopy and atomic force microscopy techniques. Our goal is to further clarify the influence of physiologically relevant metal cations on insulin aggregation and contribute to the understanding of the polymorphic nature of toxic amyloids.

104101, https://doi.org/10.1016/j.jbc.2023.104101

Abstract 2032

Multi-state Unfolding Processes: Discrimination of protein domains by urea-induced thermal shift

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Co-solute induced molecular denaturation and aggregation mechanisms related to stability changes for multi-domain proteins like mAbs are often hard to monitor experimentally. In addition, a thorough theoretical explanation is often missing. We performed intrinsic fluorescence (IF) measurements of monoclonal antibody (mAb) samples for different aqueous urea concentrations under thermal denaturation. Our results show that the denaturating effect of urea on individual mAb domains can be explained by linear mapping of the thermal shifting curve to the actual urea concentration. Notably, the achieved thermal shifting curves can be assigned to certain protein domains, which enables discrimination of overlapping denaturation processes. Our approach highlights the benefits of direct monitoring of co-solute effects on the conformational stability of mAb domains and its colloidal stability. We will discuss the experimental approach and present the corresponding outcomes in terms of the underlying molecular mechanisms.

104102, https://doi.org/10.1016/j.jbc.2023.104102

Abstract 2046

Synthesis of Microwave-bases Fibrils

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Juan Lopez-Garriga

Currently, *in vitro* fibrils are most commonly synthesized with an oven. However, this causes uneven heating and leads to fibrils of different sizes and morphology. Previous research has shown that beta-lactoglobulin fibrils can be synthesized in a microwave with less conformational heterogeneity which helps create a more representative sample when used as a model for fibril diseases. The goal of this research was to apply and optimize this microwave technique to make Lysozyme fibril with less variable fibril morphology than oven-based fibrils. To ensure homogenous solution, acid buffer was mixed with lysozyme at 25 mg/mL and carefully vortexed. After microwaving on interval sets of 10 seconds every 80 seconds, 15 uL of solution was taken out of the vial after 0,5,10, and 15 heating repetitions. The 15 uL were added to a Thioflavin T solution and later transferred to a quartz cuvette and measured change of fluorescence against stock Tht solution. Results show that the change in fluorescence was not significant and suggests that fibril were not being formed correctly. Possible explanations is that the protein was already denatured or it could be that the microwave fibril forming techniques apply to certain protein morphology at the standard conditions. For future experiments, other proteins of similar morphology to beta -lactoglobulin will be used.

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ASBMB

Abstract 2048

Enzyme-substrate interactions driving lysine deacetylase specificity

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Tasha Toro

Lysine acetylation is a common post-translational modification, but regulation of lysine acetylation is incompletely understood, in part because the activity of the enzymes regulating the modification are not well-characterized. One family of enzymes controlling lysine acetylation are the metal-dependent lysine deacetylases (KDACs), which have a conserved catalytic domain structure. Although a few specific protein substrates of some KDACs have been identified, there is little understanding of how the interactions between each KDAC and potential substrates determines the specificity of each enzyme. Our objective is to identify the interactions between selected KDACs and potential substrates that drive the specificity. Here, we present some of the short-range interactions occurring between KDAC residues and the residues adjacent to the acetyllysine residue in potential substrate peptides. Distinctive residues in each KDAC interact preferentially with particular substrate residues, and these interactions correlate with either enhanced or reduced catalytic activity. Both nonpolar residues and charged residues in peptides have interactions with each KDAC that contribute to the selectivity. Overall, we report progress toward mapping the interactions that ultimately dictate the potential specificity of each enzyme, and which will lead to a description of the pool of potential substrates for each KDAC within a cell. Identifying the characteristic interactions of each KDAC also has applications for design of molecules specifically targeting a single member of the metal-dependent KDAC family.

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Abstract 2055

Bacterial expression of unstable mutants of a human mitochondrial aminoacyl-tRNA synthetase

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The AARS2 gene codes for the mitochondrial alanyl-tRNA synthetase. Mutations in AARS2 are linked with severe diseases, including infantile cardiomyopathy and childhood to adulthood onset leukodystrophy. We are interested in understanding how molecular differences in the mutated proteins result in different disease phenotypes. One hypothesis predicts that mutations associated with more severe phenotypes will lead to greater protein instability compared to those associated with less severe phenotypes. We are interested in testing this hypothesis *in vitro* via expression and purification of the mutant and wildtype protein. Initial attempts to produce mutant AARS in a bacterial expression system were unsuccessful, with poor expression of soluble proteins. We have been able to increase mutant expression through the addition of an MBP solubility tag, which has greatly improved purification of both full-length wildtype and mutant proteins. To obtain intact full-length native proteins, TEV protease is used to remove the MBP tag.

This work was funded by Carleton College.

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Abstract 2056

The Role of Pseudophosphatase MK-STYX in HDAC6 and Dynein Dynamics

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Shantá Hinton, Lynn Zavada

The pseudophosphatase MK-STYX (mitogen activated kinase phosphoserine/threonine/tyrosine binding protein) is a member of the MAPK phosphatases (MKP) dual specificity family of phosphatases. However, MK-STYX is rendered catalytically inactive as a phosphatase because of the amino acid sequence FSX5R in its active site, instead of HCX5R. We previously reported that MK-STYX interacts with the RNA binding protein G3BP1 (Ras-GTPase SH3 domain binding protein-1) in the stress response pathway. During stress response, mRNA translation is halted, and stress granules, which are cytoplasmic aggregates of mRNA and other proteins, are formed as a protective response. G3BP1 serves as a major structural component of these stress granules. Additionally, microtubules serve as scaffolding and transport during the initial assembly of stress granules. It is known that G3BP1 is transported to stress granules in a microtubule dependent manner by a complex of 2 proteins: dynein and histone deacetylase 6 (HDAC6). HDAC6 is an atypical member of the histone deacetylase family because it is cytosolic and not nuclear. It is responsible for facilitating transport of other proteins by dynein. Additionally, it regulates microtubule dependent transport through the deacetylation of polymerized microtubules. We previously demonstrated that MK-STYX decreases stress granule assembly, however it does so independent of G3BP1 phosphorylation status at serine 149. Given the essential nature of G3BP1's interaction with the HDAC6-Dynein complex, we decided to investigate what effects if any that MK-STYX is having on the two proteins. Because HDAC6 deacetylase activity has been shown to effect stress granule assembly, we investigated whether MK-STYX had an effect on HDAC6 deacetylation. HDAC activity assay pilot studies indicate that HDAC6 deacetylase activity decreases in the presence of MK-STYX. This result suggests that MK-STYX may regulate stress granule assembly by altering HDAC6 activity. To further understand the interaction between MK-STYX and Dynein, we transfected HEK-293 cells with fluorophore conjugated constructs and studied their localization. Dynein appears to colocalize in the presence of MK-STYX and form aggregates. We have previously demonstrated that MK-STYX does not localize to stress granules, so these dynein aggregates may be a novel way of controlling their activity. These are exciting results which we are continuing to explore.

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Abstract 2059

Structural Modeling and Analysis of [beta]-amylase9

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Karen Siddoway, Jonathan Monroe, Christopher Berndsen

Starch, a polysaccharide glucose chain, is the food that plants make for themselves, and the plants it comes from help make up part of the global food source. The β-amylase family is a group of proteins that catalyze starch degradation processes. β-amylase9 (BAM9) is found in starch-containing plants, including *Arabidopsis thaliana*, and has been linked to the regulation of starch production in them. Even so, there is very little known about how BAM9 actually affects the starch degradation process. BAM9 is also currently thought to be in the class of pseudoenzyme, as it has thus far not been shown in literature to have any catalytic activity because it lacks a key residue essential for catalytic activity in other BAMs. The ∝-amylase (AMY3) is a related enzyme that has been shown to be catalytically active in the starch degradation processes in plants. Recent yeast two-hybrid assays and size exclusion chromatography experiments have suggested an interaction between BAM9 and AMY3. It is unclear why and where BAM9 and AMY3 interact and a better understanding of how this protein affects the process of starch regulation is important for the future of food stability. There are currently no structures of BAM9 or known functions of it within the starch degradation process. To address this issue, a model structure of BAM9 was generated using AlphaFold2 in order to predict possible binding sites and interactions with other molecules involved in the starch degradation process. We then performed a series of OpenMM molecular modeling simulations using this proposed structure to describe the dynamics of the protein and how interacting partners affect these motions. As BAM9 showed potential regulatory activity with AMY3, interactions of BAM9 with AMY3 were modeled to show how these proteins interact and suggest a possible activation mechanism. Two potential interaction sites were identified by conservation and are being tested experimentally. These data will provide the structural basis for a regulatory mechanism by BAM9 for AMY3 mediated starch degradation.

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ASBMB

Abstract 2073

Resolving substrate specificity of metal dependent lysine deacetylases

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Lysine acetylation is an important post-translational modification found on thousands of proteins. Lysine deacetylases (KDACs) are responsible for removing acetylation, and this reversible modification serves as an important regulatory mechanism in cells. There are 11 human metal dependent KDACs which share a conserved active site. While there are thousands of acetylated proteins in human cells, very few direct enzyme-substrate pairs have been identified. To better understand the role of KDACs in cellular processes, we need to understand the specificity of individual KDACs. Although there is a great deal of conservation near the active site, we hypothesize that small structural differences between family members lead to differences in substrate preference. We have developed a fluorescence based assay as well as a MALDI-TOF mass spectrometry based assay to measure deacetylation of peptide substrates *in vitro*. First, we were able to observe weak but measurable activity of class IIa KDACs with biologically relevant peptides. We have characterized the activity of representative metal-dependent KDACs from each class with acetylated peptides derived from known human acetylated proteins. Analysis of the activity data revealed statistically significant preferences for each enzyme for the positions flanking the acetyllysine. From these data, we can identify differences in specificity both within and between KDAC classes. Molecular dynamics simulations provide insight into how each KDAC interacts with its preferred substrates. These data will ultimately be useful for understanding how KDACs mediate cellular processes and function in disease progression, and may also be instrumental for designing more targeted KDAC inhibitors.

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Abstract 2074

KDAC-Substrate Specificity in Cellular Context

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Lysine acetylation is a post-translational modification that occurs on human proteins. The presence of acetylation on these proteins is important for the development of diseases; therefore, deacetylation is also important. Lysine deacetylases (KDACs) are a metal-dependent enzyme family that is responsible for reversing lysine acetylation. To gain insight into how two members of this family, KDAC6 and KDAC8, select substrates to deacetylate, we hypothesized that KDAC activity with peptides derived from putative substrate proteins would predict activity with the corresponding full-length protein. Using an *in vitro* fluorescence-based assay, we determined that several such peptides are substrates of each KDAC. However, the peptides derived from the putative substrates were not always predictive of activity of the full-length protein with KDACs. To determine which KDAC is responsible for deacetylation of the corresponding full-length protein in their biological context, we utilized cell-based studies that detect changes in acetylation status of target proteins dependent on cellular KDAC activity. The effect on acetylation level is a result of manipulating KDAC activity levels within the cell to determine a correlation between potential full-length proteins and KDACs within their biological context, e.g., if a full-length protein is a putative substrate of a particular KDAC, then we expect to detect an increase in acetylation. After detecting acetylation changes in a lysate containing target protein either endogenously or overexpressed, target proteins are then reacted with a recombinant KDAC *in vitro* to determine whether the enzyme is capable of deacetylating the full-length protein, and the amount of acetylation assessed by immunoblotting and/or mass spectrometry. Overall, we observed that only some putative substrates previously attributed to certain KDACs show evidence of a direct deacetylation by a particular KDAC. Identifying direct targets of KDACs is important for understanding the specificity of each KDAC in a biological context, and will ultimately contribute to our understanding of the role of KDACs in disease.

We gratefully acknowledge the National Institutes of Health (R15GM129682, G12MD007595, and UL1GM118967), the National Science Foundation (CHE 1625993 and MCB 1817358), the U. S. Army Research Laboratory and the U. S. Army Research Office (W911NF1910452), and the Louisiana Cancer Research Consortium.

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Abstract 2094

Investigating Metal Binding to KmtR

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Mycobacterium tuberculosis (*M. tuberculosis*), the causative agent of tuberculosis, infects nearly one-third of the world's population and is responsible for the death of almost two million people annually. The bacteria encodes several metal transport systems that are critical for its survival in phagosomes where the metal concentrations are always changing. The bacteria has two metalloregulators that are associated with nickel and cobalt export, NmtR and KmtR. Nothing is known about why KmtR is responsive to Ni(II) and Co(II) binding and if KmtR is capable of binding to other first-row transition metals.The fact that *M. tuberculosis* has two regulators that are associated with the expression of two different exporters for Ni (II) and Co(II) suggests that maintaining the intracellular Ni(II) and Co(II) concentrations is critical to the bacteria. The metals site structure for the cognate metal Ni(II) and the noncognate metal Zn(II) have been determined by X-ray Absorption Spectroscopy. Metal binding studies conducted using Isothermal Titration have determined that KmtR binds to Ni (II) and Co(II) with nanomolar affinity.

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Abstract 2101

Modulating Alzheimer's Disease by mTORC1 inhibition to augment lysosomal activity

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Pathogenic lesions in the central nervous system, comprised of insoluble protein aggregates such as the microtubule associated protein, tau, are thought to be key in the pathogenesis of Alzheimer's Disease. Protein aggregates are naturally degraded through the autophagy-lysosomal pathway; promoting the clearance of tau has emerged as a potential therapeutic avenue. Key in control of the auto-lysosomal pathway is the activity of the mechanistic target of rapamycin (mTOR) complex 1 system. The mTORC1 system functions by sensing of nutrient status to control cellular metabolism, and directly inhibits the auto-lysosomal pathway. Importantly, mTORC1 activity is increased in brain tissue of AD patients and is associated with tau level; there is a concurrent decrease in autophagic activity. As direct mTORC1 inhibition results in pleiotropic effects and toxicity, an alternative means to influence its activity comes through mTORC1 Regulators, a collection of 30+ recently characterized proteins directly upstream of mTORC1 that modulate its activity. Here we show upregulation of a key mTORC1 inhibitory protein, KPTN, impairs mTORC1 activity and aids in clearance of tau protein in neuronal cell culture. Through unbiased siRNA screening we identify that KPTN is potently controlled by the E3 ubiquitin ligase PDZRN3, leading to KPTN ubiquitination and degradation. Moreover, KPTN and PDZRN3 protein levels are inversely correlated in aged mouse brains, suggesting a potentially causal relationship. Further, we have developed a class of small molecule KPTN activator compounds, which prevent mTORC1 activation, increase lysosomal activity, and aid in tau protein clearance. The net effect of KPTN aids in clearance of toxic protein aggregates by activation of autophagy, and suggest an alternative pathway of autophagic regulation for tau clearance.

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ASBMB

Abstract 2115

Cryo-EM structures explain why Rad24-RFC preferentially loads the DNA checkpoint 9-1-1 clamp to DNA regions with a single strand gap size of over 10 nucleotides

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The PCNA clamp is a processivity factor of DNA polymerases and is loaded onto a 3′-recessed DNA end by the RFC complex, while the 9-1-1 clamp functions as a DNA damage checkpoint signal and is loaded onto the 5′-recessed DNA end by the alternative clamp loader Rad24-RFC. Recent work by us and others have showed that both RFC and Rad24-RFC contain an internal chamber DNA binding site and an external shoulder DNA binding site that can accommodate the 3′- and 5′-recessed ends, respectively. However, RFC can load PCNA onto a DNA of any gap size by virtue of its ability to unwind the duplex from the 3′-recessed end inside the chamber, but Rad24-RFC cannot load the 9-1-1 clamp at a nick, but does load 9-1-1 onto DNA with a gap size of 9 nucleotides (nt), although the underlying mechanism has been unclear. In our presentation, we will show two cryo-EM structures of the Rad24-RFC-9-1-1 complex, one bound to a 10-nt gapped DNA and the other bound to a 5-nt gapped DNA. By comparing the two structures, we found that Rad24-RFC can comfortably accommodate two dsDNA segments when the ssDNA linker is about 10-nt or longer. However, we discovered that Rad24-RFC does not unwind duplex DNA. In order to fit the 5-nt gapped DNA in the Rad24-RFC, the 5-nt ssDNA linker is stretched straight, and the dsDNA segment at the 3′-recessed end is rotated by 180° compared to the 10-nt gapped DNA in order to minimize the distance between the two dsDNA segments. Therefore, our work explains why Rad24-RFC preferentially loads 9-1-1 clamp onto larger gaps and reveals a novel cellular strategy to differentially utilize the PCNA and 9-1-1.

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Abstract 2123

Genetic Therapies for Spinal Muscular Dystrophy: A Study of Mutations and Deletions of the SMN1 Gene

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Spinal Muscular Atrophy (SMA) is a recessive neuromuscular disorder that is characterized by the degeneration of the alpha motor neurons. The Tudor domain of the spinal motor neuron 1 (SMN1) gene allows for the binding of multiple proteins to form a spliceosomal core complex responsible for splicing introns and exons to form proteins within motor neuron axons. Mutations within SMN1— located on chromosome 5q13.2– leads to interference of the expression of the SMN protein, resulting in a compromised spliceosomal complex structure. The most common mutation is a complete deletion of SMN1, accounting for 95% of SMA patients. The remaining 5% can be credited to nonsense, missense, splicing, and frameshift mutations throughout different regions of the gene, including the Tudor domain. Using Jmol protein visualization software we will model the structure of the SMN protein to highlight these significant mutations in order to better understand research into current genetic therapies focus on increasing expression of the nearly homologous SMN2 gene with the goal of reducing the effects of SMA.

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Abstract 2124

Disease-Associated Non-Coding Mutations Alter NKX2-5 Biding Affinity to Their Specific Binding Site

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Transcription factors (TFs) are proteins that bind to specific non-coding regulatory DNA to regulate gene expression and control biological processes. For example, NKX2-5 is a tissue-specific TF involved in heart development. Over 90% of disease-associated variants occur in the non-coding regions of the genome and can alter TF recognition of their specific binding site and dysregulate gene expression. Previously, we computationally identified more than 900 mutations affecting NKX2-5 binding using a Position Weight Matrix (PWM) – based predictive model (SNP2TFBS) and prioritized five variants that are predicted to have the largest impact in binding. A positive ΔPWM score predicts an increase in binding affinity; a negative ΔPWM score predicts a decrease. We chose five disease-associated variants predicted to affect NKX2-5 binding for *in vitro* validation: rs7350789 (ΔPWM = 258), rs61216514 (ΔPWM = − 232), rs7719885 (ΔPWM = − 212), rs3892630 (ΔPWM = 146) and rs747334 (ΔPWM = − 187). Using Electrophoretic Mobility Shift Assays (EMSA), we validated our computational predictions by observing changes in NKX2-5 binding to the tested variants. We quantified these changes in affinity by determining apparent dissociation constants (Kd, app) between variants alleles. Variants rs7350789, rs7719885, rs747334, and rs3892630 showed an increase in binding affinity resulting in the decrease of Kd,app. Variant rs61216514 showed a decrease in binding affinity resulting in the increase of Kd,app. The experimental data demonstrated that the predictions of rs3892630, rs61216514, and rs7350789 were correct. The next step is to evaluate how they can impact gene expression through reporter assays. Successful completion of this project will help us understand how these mutations can result in harmful phenotypes. This can be decisive in preventing diseases through treatment and early diagnosis through genetic testing.

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Abstract 2133

Engineering of protein-stabilizing surface ion pairs using Arg and Glu residues

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Naturally-occurring surface ion pairs have been identified as a factor in the stability of proteins from thermophilic and hyperthermophilic organisms. In this study, single substitutions of Arg or Glu engineered at position 50 or 59 in a hyper-stabilized mesophilic staphylococcal nuclease protein were destabilizing, based on thermal denaturation by fluorescence spectroscopy. However, double substitutions at the same positions to produce either Arg-50/Glu-59 or Glu-50/Arg-59 variants increased the melting temperature, but to different extents. The substitution sites are positioned on alpha helix 1 and the preceding loop, with a Ca – Ca distance of 5.6 Å in the background protein crystal structure. X-ray crystallography studies were initiated to detail the single-substituted proteins and putative ion pairs, including the steric constraints imposed by the engineered residues and the effects of charge reversal. Complementary fluorescence studies were also performed to assess ionic strength and pH effects. The results of these studies reveal an adaptable design capable of modulating protein stability through surface amino acid substitutions.

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ASBMB

Abstract 2134

Development of Purification Protocols for the Aggregation-Prone Transactivation Domains of the EYA Transcriptional Regulators Jaclyn N. Remsing*, Steven T. Whitten, Karen A. Lewis

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Steve Whitten, Karen Lewis

Members of the Eyes Absent (EYA) family of transcriptional regulators are critical gatekeepers for cellular response to environmental stressors, including the decision between proliferation and apoptosis in response to DNA damage. These regulators have been implicated in immune function, organogenesis, and tumorigenesis. EYA proteins are comprised of two domains: a well-characterized, folded phosphatase "enzymatic domain" (ED) and a less-characterized, intrinsically disordered "transactivation domain" (TAD). A recent analysis of the human proteome using ParSe, a computational algorithm that predicts phase separation behavior, identified potential phase-separating regions in the TADs of all four human EYA proteins. However, phase-separating activity of these domains has not been observed experimentally. We hypothesize that the EYA proteins undergo phase separation through the N-terminal TADs, and that this activity directly regulates the protein's function as a transcriptional regulator. Phase separating proteins have a tendency to aggregate, and so present challenges for recombinant expression and purification. Currently, we are developing methods to purify these proteins while maintaining solubility. The TADs of EYA1 and EYA2 are robustly expressed in *E. coli* as cleavable His-tagged proteins. Our current challenge is to identify buffer conditions that will keep EYA-TAD soluble and monomeric, while still allowing for metal-affinity chromatography and protease cleavage of the His tag. The EYA TADs remain soluble in as little as 2.5 M urea, and preliminary data suggest that the urea might be able to be further reduced when pH is increased above physiological levels. The purification of Eya TADs will allow for characterization of both protein structure and phase separation behavior using protein NMR, analytical size exclusion chromatography, and turbidity assays.

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Abstract 2135

Structural Impacts of Phosphorylation of the KCNQ2 B Helix on CaM and PIP2 Binding

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KCNQ produces the M-current known to regulate neuronal firing. These tetrameric channels are inhibited by second messengers activated by G protein coupled receptors (GPCR) such as the muscarinic receptor M1R. Such inhibition of the M-current results in increased neuronal activity. Thus, precise control of these channels is necessary to maintain healthy neuronal activity. Calmodulin (CaM), phosphatidylinositol 4,5-bisphosphate (PIP2) and protein kinase C (PKC) are GPCR signaling factors that interact with KCNQ channels to modulate the M current. Stimulation of GPCR's, such as the muscarinic receptor M1R, lead to the hydrolysis of PIP2 that results in decreased M current. Ca2+ released by GPCR signaling binds to CaM and PKC to facilitate further inhibition of M-current. KCNQ channels are comprised of a combination of KCNQ1-5 protein subunits. Each subunit has two CaM binding domains, called the A and B helices, that are surrounded by several PIP2 binding sites. Ca2+-bound CaM embraces the A and B helices to suppress M-current. Some studies support that in low [Ca2+], only the B helix interacts with apoCaM, although this is still a point of debate. Therefore, the role of PIP2 during the reconfiguration of CaM with each KCNQ subunit is unclear. It was recently shown that PIP2 can bind the B helix in the presence of apoCaM, and high [PIP2] causes Ca2+/CaM to dissociate from KCNQ1. In contrast, high Ca2+/CaM competes with PIP2 for binding KCNQ1. Yet, functional studies indicate a lack of PIP2 binding the B helix of KCNQ3, despite the high sequence similarity. These discrepancies may arise from selective PKC phosphorylation of the B helix. The proposed PIP2 binding site within the B helix is part of a PKC sequence recognition motif, so mutating this region may interfere with both PIP2 binding and phosphorylation of the B helix. KCNQ2 has two PKC-targeted serines within its B helix, whereas KCNQ3-5 have one PKC-targeted threonine, and KCNQ1 bears none. This study tests the hypothesis that phosphorylation of the B helix of KCNQ2 reduces its interactions with CaM and PIP2. We use NMR and other biophysical methods to establish a model to clarify the role of phosphorylation in coordinating CaM and PIP2 for regulating KCNQ channel activity.

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Abstract 2137

Structural and biophysical characterization of the *Vibrio cholerae* ferrous iron transport protein B (FeoB)

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The acquisition and transport of ferrous iron (Fe2+) is essential for the survival and the virulence of many infectious prokaryotes. While bacteria possess several methods to acquire Fe2+, the ferrous iron transport (Feo) system is the most important Fe2+ transport complex, and the Feo system has strong ties to bacterial pathogenesis. The most conserved component of the Feo system is FeoB, a polytopic transmembrane protein containing a soluble N-terminal domain (termed NFeoB) that has been shown to have GTP hydrolysis activity. Intriguingly, some studies have revealed that a select number of FeoBs hydrolyze both GTP and ATP, making them NTPases rather than strict GTPases. While sequence analyses suggest key differences between GTPase and NTPase FeoBs, there is a lack of structural information defining the nucleotide promiscuity of these G-protein like domains. In this work, we report the crystallization of apo *Vibrio cholerae* NFeoB (VcNFeoB), which was previously defined as an NTPase. Comparisons to other GTPase and NTPase type NFeoBs reveal key differences that are hypothesized to play a role in nucleotide discrimination. Additional biophysical analyses such as isothermal titration calorimetry (ITC) and NTPase activity assays are used to characterize VcNFeoB further. These results give insight into ferrous iron acquisition of this problematic pathogen, which could be leveraged for future therapeutic developments.

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Abstract 2144

Rapid Analysis of Small Angle X-ray Scattering Data in Python

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Ruby Adkins, Christopher Berndsen

Everyone who has taken a Biochemistry class has heard the phrase structure determines function. Small-angle X-ray Scattering (SAXS) is a method that measures the angle of X-ray scattering from a sample to gain data on the structural information such as size, shape, and secondary structure. This structural information can give a good indication of the function of the biomolecule investigated and may be useful for development of therapeutics targeting these molecules. The SYBILS beamline at Advanced Light Source in California collects high throughput SAXS and SEC-SAXS data for users from all over the country. The high throughput ability of the beamline allows it to rapidly acquire large amounts of data that may be useful for drug discovery and studies of biomolecules in many conditions. Users currently receive un-analyzed data and they need to manually work through the data to decode if the data are not good or when the sample was damaged by the X-ray beam. This process is time consuming and requires a reasonable level of comfort with working with SAXS data. Because the beamline collects replicates on the same sample, it is also helpful to know how these values change as the replicates are collected to indicate if the sample was damaged by the X-ray beam during the experiment. This can tell the user whether it is a good data set to use or if it was damaged too quickly. Thus, an automated system could help scientists know where to preliminarily focus their efforts. We are writing software to analyze high-throughput SAXS data for the SIBYLS beamline which will guide the user in their analysis. We have written a program using Python, called "Rgifier," that will analyze the data and calculate standard values that determine size and shape such as, the radius of gyration, mass, Porod values, volume, and shape, along with the error of these calculations. The software writes the results into a summary.csv file that has the values of interest and an indication of if the biomolecule is elongated or condensed. We have tested the code on small standard data sets and larger experimental data sets. The code can currently run 7.5 seconds per well while manually fitting in RAW takes about 120 seconds per well when done by an expert user. The code has been shown to be able to detect radiation damage across frames in datasets. We analyzed protein and non protein biomolecules in Rgifier and compared it to manual fitting. This showed that the Rgifier can produce accurate values of interest for different types of biomolecules. Currently, we are optimizing the code parameters to maximize accuracy on protein and non protein datasets while preparing it for use by the beamline. Our preliminary code will enhance the rate at which scientists can describe the breadth of biomolecule structure and lead to a greater ability to address the problems of the future.

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Abstract 2145

Structural and functional studies of nucleic acid binding domain from rabbit SAMD9

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Human sterile alpha motif domain 9 (SAMD9) is a cytosolic protein that plays an important role in antiviral response and tumor suppression. Mutations in SAMD9 results in either the gain of function or the loss of function, resulting in multisystem developmental disorders including many pediatric myelodysplastic syndromes. SAMD9 is a multidomain protein whose domain structure and molecular functions is largely unknown. We previously reported that human SAMD9 effector domain binds to double stranded (ds) nucleic acid (NA) which is important for its anti-viral and anti-proliferative activities. We identified certain key residues from human SAMD9 effector domain that are essential for its ds NA binding and function. Interestingly, rabbit SAMD9 lacking one key residue but it is fully functional *in vivo*. Our *in vitro* gel shift assays also show that rabbit SAMD9 binds ds NA in 2:1 molar ratio. Hence, we hypothesize that rabbit SAMD9 binds ds NA with a different mode, that is why we want to study the structure of rabbit SAMD9 with ds NA. To investigate the mechanism of double stranded nucleic acid binding by rabbit SAMd9 we are carrying out structural analysis by X-ray crystallography. Fully revealing the atomic details of rabbit SAMD9 binding with nucleic acid will provide new insights for its structure and function.

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Abstract 2157

Increases in Physiologic ROS During Glucose Limiting Conditions or from SOD1 Deletions Result in Translational Repression through No-Go Decay and P-body Assembly

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Emily Cianflone, Anna Jorgensen, Gretchen McManus, Amber Lobb, Autumn Barloon

When glucose is abundant, the budding yeast *Saccharomyces cerevisiae* preferentially metabolizes it anaerobically to allow for rapid growth as well as to minimize the amount of intracellular reactive oxygen species (ROS). However, as glucose becomes depleted, yeast will enter diauxic shift. Under this condition, growth will slow and there is a switch to aerobic glucose metabolism. This will, in turn, be accompanied by an increase in ROS, which can be damaging to nucleic acids and affect gene expression. Previous work showed that increases in intracellular oxidation from exposure to nonphysiologic sources of ROS can lead to formation of 8-oxoguanine bases (8-oxoG) in mRNA. The presence these damaged guanines can elicit ribosome stalls, resulting in translational repression of these damaged mRNA, the activation of the no-go mRNA quality control pathway, and a subsequent increase in Processing body (P-body) assembly. No-go decay is mediated by the proteins Hbs1p and Dom34p, which act to remove stalled ribosomes, and will promote cleavage of the mRNA at the stall site. The remaining 5′ fragment is subject to 3′-5′ decay by the cytoplasmic exosome. Meanwhile, the 3′ fragment is degraded 5′ to 3′ by the exonuclease Xrn1p. At current, it is an ongoing question as to whether mRNA damage and no-go decay activation occurs in response to physiologic sources of ROS. Upon growing yeast into diauxic shift, an increase in P-body assembly was observed. P-bodies contain mRNA decay enzymes and are sites where nontranslating mRNA can localize to after they are translationally repressed. This observed increase in P-body assembly appears to be due to upregulation of no-go decay, as P-body assembly is reduced in strains lacking either Dom34p or Hbs1p. Upon exposure to the antioxidant quercetin during diauxic shift, P-body assembly is abrogated showing that generation of ROS is responsible for the activation of no-go decay and subsequent P-body assembly. To ensure that the increase in P-body assembly is due solely to low glucose conditions, the same result can be phenocopied by exposing yeast to conditions in which glucose is limiting (0.5% glucose). As expected with the increased oxidation from glucose limiting conditions, global translation is also reduced. Interestingly, strains lacking the cytoplasmic Sod1p show a reduction in global translation, along with an upregulation of no-go decay and P-body assembly. Additionally, a dom34Dsod1D strain shows significant growth defects on media with limited glucose. These data taken together indicate that increases in physiological derived ROS can damage mRNA leading to activation of No-Go Decay and subsequent P-body assembly.

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ASBMB

Abstract 2159

Deciphering the Mechanism of Ubiquitin Transfer by the Non-Canonical E2 Conjugating Enzyme, Ubc6

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Karen Dunkerley, Rachel Klevit

A series of enzymes form a transfer cascade to maneuver the small, 8 kDa ubiquitin (Ub) molecule to its final place on select substrates, resulting in a variety of cellular events including signalling and protein degradation. Ub transfer is initiated by an E1 activating enzyme, which then passes the Ub to one of ~40 different E2 conjugating enzymes (E2~Ub). The final attachment of Ub onto a substrate is mediated by one of over 600 E3 ubiquitin ligases (E3). Though the E3 is an important bridge between the activated Ub and substrate, the E2 is now considered the 'decision maker' in the final Ub location, linkage type and chain length. At a structural level, the majority of E2s contain a highly conserved, 150-residue UBC domain with a conserved catalytic site poised for accepting Ub from the E1. Most of the characterized E2s are able to transfer Ub to lysine; three E2s, to date, transfer Ub to non-lysine sites (non-canonical) including cysteine, serine/threonine, and free N-termini. Despite these classifications, the precise molecular mechanisms of Ub transfer are still unknown. The goal of this research is to use the non-canonical E2 Ubc6 in both structural and functional studies to precisely map the important residues required for Ub transfer. Ubc6, the yeast homolog to human Ube2J2, is an endoplasmic reticulum membrane bound E2, which in addition to the UBC domain, contains a long C-terminal disordered region followed by the transmembrane helix. Ubiquitylation assays reveal that Ubc6 is readily charged with Ub and that Ub is subsequently discharged onto Ubc6 itself, even in the absence of the partner E3, Doa10. Base treatment, but not reduction of the resulting autoubiquitylated Ubc6 cleaves the attached Ub indicating the linkage occurred on serine, threonine and/or tyrosine residues in Ubc6. Mutation of numerous residues surrounding the catalytic site do not affect charging but have varied effects on Ub discharge, implicating a role of these sites in the reaction chemistry of Ub transfer. Autoubiquitylation was not observed in a Ubc6 construct lacking the C-terminal disordered domain. Structural techniques including nuclear magnetic resonance and cross-linking mass spectrometry reveal the positioning of a conjugated Ub molecule required for successful Ub transfer. This work reveals the key residues involved in the mechanism of Ub transfer onto serine/threonine/tyrosine residues employed by Ubc6 and how the disordered C-terminal tail is positioned. Overall, these results offer critical insight into how small changes across this highly conserved family of enzymes have drastic effects on the linkage type and location of E2-mediated Ub transfer.

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Abstract 2161

Novel Link between Ire1 and MAPK Slt2 Pathways via Transcription Factor Rlm1 in Yeast *Saccharomyces cerevisiae*

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Jagadeesh Uppala, Cing Kiim, Madhusudan Dey

Adverse pathophysiological conditions and external stresses may cause protein misfolding, unfolding, and aggregation inside the endoplasmic reticulum (ER), a state called ER stress that disrupts cellular protein homeostasis, or proteostasis. To restore disturbed ER proteostasis, cells evoke adaptive signaling networks, specifically activating the ER-resident protein kinase Ire1 and mitogen-activated protein kinase (MAPK) Slt2 in Yeast *Saccharomyces cerevisiae*. The active Ire1 cleaves HAC1 mRNA at two sites to remove an intron. The spliced HAC1 mRNA encodes an active transcription factor that activates the expressions of protein folding enzymes and chaperones to increase the folding capacity of cell. In contrast, MAPK Slt2's role in ER proteostasis has persistently been a mystery, though it is popularly known to initiate the cell wall integrity (CWI) signaling pathway by phosphorylating the transcription factor Rlm1. We observed that yeast cells lacking Rlm1(*rlm1Δ*) or both Rlm1 and its paralog Smp1 (*rlm1Δsmp1Δ*), is moderately sensitive to ER stressor tunicamycin (0.25 μg/ml), suggesting that Rlm1 could play a role in ER proteostasis. To investigate the role of Rlm1 in ER proteostasis, we grew wild type (WT) and *rlm1Δsmp1Δ* strains in the presence and absence of ER stressor DTT for 2 and 4 hours. From those cells, we isolated both RNA and protein and monitored splicing of HAC1 mRNA by reverse transcriptase-PCR (RT-PCR) and expression of Hac1 protein by Western blot analysis. Compared to wild type (WT), a significant reduction in spliced HAC1 mRNA splicing (~8-fold) was observed in the *rlm1Δsmp1Δ* strain grown with DTT for 4 hours. We also observed a significant reduction in HAC1 mRNA splicing (~8-fold) in the *slt2Δ* strain grown with DTT for 4 hours. Consistently, Hac1 protein expression was reduced after 4 hours in the *rlm1Δsmp1Δ* strain. These results suggest that adaptation to prolonged ER stress requires Rlm1 function. Rlm1 is a MADS-box transcription factor that binds to a conserved 5′-CA(A/T)6G-3′ DNA element at the promoter and activates transcriptions of several genes. Therefore, we hypothesize that Rlm1 might bind to the Ire1 promoter and modulate IRE1 transcription. To test our hypothesis, we monitored expression of the LACZ gene from the IRE1 and the HAC1 promoter. Interestingly, we observed a decrease in

the LACZ expression (1.5-fold) from the IRE1 promoter in the *rlm1Δsmp1Δ* strain compared to its isogenic WT strain. We did not see any difference in LACZ expression from the HAC1 promoter. Taken together, our data suggests that during prolonged ER stress, MAPK Slt2 activates Rlm1 that, in turn, binds to the IRE1 promoter to enhance the IRE1 expression.

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Abstract 2172

Cyclic Tetra-Adenylate (cA4) Recognition by Csa3a; Implications for an Integrated Class 1 CRISPR-Cas Immune Response in *Saccharolobus solfataricus*

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Class I CRISPR-Cas systems, primarily Type I and Type III, are the most abundant CRISPR systems in archaea and bacteria, but the mechanisms behind the immune response regulation are not well understood. Csa3 family transcription factors, composed of an N-terminal CARF domain and C-terminal winged helix-turn-helix domain, are often associated with Type I CRISPR-Cas systems in the order Sulfolobales. Csa3 transcription factors are hypothesized to be controlled by cyclic oligoadenylate (cOA) second messengers produced by Type III surveillance complexes, and this interaction is predicted to modulate their DNA-binding activity. We therefore investigated the predicted interaction between Csa3a and cyclic tetra-adenylate (cA4). Isothermal titration microcalorimetry showed *S. solfataricus* Csa3a binds cA4 at a biologically relevant dissociation constant of 1 μM in an entropically driven interaction. Ring nuclease assays revealed Csa3a lacks the self-regulatory ring nuclease activity of other CARF domain proteins. This allowed us to crystallize and solve the structure of the Csa3/cA4 complex. The structure revealed conserved motifs responsible for cA4 binding and illuminated significant conformational changes resulting from the interaction. We also identified a consensus 18-bp palindromic motif based on the *S. islandicus* Csa3a binding site reported by Liu et al. (Nucleic Acids Res. 45:8978). This motif, which we designated CAPPa, is conserved in the 27 sequenced members of the order Sulfolobales, and shows synteny with Csa3a in these genomes. While we found that Csa3a binds CAPPa and other promoter sequences, it does so in a nonspecific, cooperative, and cA4-independent manner. This suggests a more complex method of transcriptional regulation than previously hypothesized. Nevertheless, the interaction between Csa3a and cA4 demonstrated here represents a nexus between the Type I and Type III systems in *S. solfataricus*; we thus propose a model where this interaction coordinates the two branches of an integrated immune system to mount a synergistic, highly orchestrated, adaptive immune response.

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Abstract 2176

Molecular basis of DNA recognition by the HMG-box-C1 module of Capicua

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The transcriptional repressor Capicua (CIC) is a key protein in cellular and developmental processes, and the mutation or improper regulation of CIC has been implicated in cancers. Two domains of CIC, the HMG-box and C1 domain, are required for the physiological binding to DNA, but how these domains cooperate and interact with the consensus recognition sequence, 5′-TGAATGAA-3′, has yet to be fully elucidated. Here, we report a 2.95-Å crystal structure of a modified CIC construct (CICmin), comprised of the HMG-box and C1 domain linked by a minimal linker region, in complex with an 18-mer DNA fragment containing the consensus recognition sequence. This structure provides means for determining the relation between the HMG-box, C1 domain, and their cooperation in binding DNA. Molecular dynamics simulations were used to provide support for the observed binding mode in the crystal structure, and functional binding assays were performed to determine the effects of cancer-associated mutations of CIC on DNA binding. Our results reveal the unique binding mode of the transcription factor CIC to its consensus sequence that involves the HMG-box and C1 domain, which adopts a helix-turn-helix (HTH) motif. The HMG-box and C1 domain form interactions with the minor and major grooves, respectively, inducing an ∼66° bend in the DNA fragment. Therefore, CIC represents the first identified example of an HMG-box protein utilizing a HTH domain within the same polypeptide for binding a consensus DNA sequence. These structural and binding studies illustrate how cancer-associated mutations may affect the binding of and affinity for DNA at the consensus recognition sequence.

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Abstract 2181

Caspase family as a paradigm for understanding the nature of protein evolution

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Caspases are a family of enzymes that are essential for regulating cellular homeostasis, namely apoptosis and inflammation. Important for initiating and executing the molecular cascades of events that lead to apoptosis and inflammation, the caspase family of enzymes has evolved into initiator and executioner classes. To date, 12 caspases have been identified in humans, which can be categorized as either executors or initiators. Caspases have evolved distinct activation and regulatory mechanisms in a cell. Caspases are a great model system for examining the principles of molecular evolution as they pertain to the tailoring of conformational free energy landscape and function. Here, we performed all atom molecular dynamics simulations on modeled structures of extant and ancestral caspases using the available monomeric, dimeric inactive, and active PDB structures. Essential dynamic simulations have demonstrated that evolution has favored the reduction of the free energy landscape, essentially tailoring the available conformations for specific activity and regulation. Executioner caspases readily form dimers, whereas initiator caspases are monomers and require activation platforms to dimerize. The principal component analysis of all caspases modeled with monomeric conformation reveals that executioner caspases sample a broader conformation than initiators, which could be why executioners can sample dimeric conformations and readily form dimers under phycological conditions. We also studied networks of the entire family to identify regularly spaced conserved residues in the core of the structure that have high degree and betweenness centrality, indicating that evolution is tweaking other residues while maintaining these conserved residues which are essential for the fold and information flow in proteins.

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Abstract 2185

Determining yeast prion propagation abilities of *Arabidopsis thaliana* Sis1 and Hsp104 orthologs

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Bridget Corpus, Harshil Bhavsar

Protein aggregation in the form of amyloid is associated with neurological diseases such as Alzheimer's disease and Parkinson's disease. Amyloids also serve as the structural basis for mammalian prions, which are infectious, self-propagating ordered aggregates of functional protein. Prions are found in the baker's yeast *Saccharomyces cerevisiae* where they propagate to progeny cells through the action of molecular chaperone proteins. Three chaperones are fundamentally required for the propagation of the prions [PSI+] and [RNQ+] in *S. cerevisiae*. These are the disaggregase Hsp104, the Hsp70 Ssa, and the J-domain protein Sis1. In the model plant *Arabidopsis thaliana*, we previously identified six functional orthologs of Sis1 and demonstrated their differing abilities to propagate distinct yeast prions when complementing a deletion of SIS1. The overall goal of this investigation is to compare amino acid sequences and structural characteristics between multiple orthologs to better understand the prion-specific propagating functions of chaperone proteins. Here we show that the J-domain and glycine-rich domains of these orthologs are fully sufficient for their prion-propagating properties, dramatically reducing the sequence complexity that must be untangled to understand the biochemical basis of their prion-specific propagation functions. Additionally, we are also examining the ability of the functional ortholog of Hsp104 found in *A. thaliana*, Hsp101, to potentially replace Hsp104 in prion propagation. Hsp101 has already been shown to successfully cooperate with yeast Hsp70s to replace Hsp104 function in thermotolerance assays. Prion propagation by Hsp101 has never been successfully demonstrated in the literature, however, this may be due to the sensitivity of prions to both over- and underabundance of Hsp104 function. Here we examine three different expression systems with the intention to vary Hsp101 expression levels to determine if any amount of Hsp101 is capable of stable prion propagation. Preliminary results so far continue to indicate that Hsp101 may not be capable of replacing Hsp104 in the yeast system to propagate either [PSI+] or [RNQ+], although experiments to test this further are ongoing.

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Abstract 2189

The Impact of N-butyldeoxynojirimycin and 1-deoxymannojirimycin on SARS-CoV-2 S1 Subunit and Angiotensin-Converting Enzyme 2 Interaction in Intestinal Epithelial Cells

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) targets mainly the respiratory tract. In addition to respiratory symptoms, many extrapulmonary manifestations were observed in the gastrointestinal tract and reported by SARS-CoV-2 patients, including abdominal pain, nausea, and diarrhea. SARS-CoV-2 binds initially to angiotensin-converting enzyme 2 (ACE2) on the host cell surface via its spike (S) protein before it undergoes endocytosis and fusion with the lysosomal membrane. The spike protein of SARS-CoV-2 is a heavily N- and O-glycosylated trimer. Glycosylation is an essential posttranslational modification in the life cycle of membrane and secretory proteins that affects their structural and functional characteristics as well as their trafficking and sorting patterns. This study aimed at elucidating the impact of glycosylation modulation on the trafficking of both S1 subunit and ACE2 as well as their interaction at the cell surface of intestinal epithelial cells. For this purpose, the S1 protein was expressed in COS-1 cells and its glycosylation modified using N-butyldeoxynojirimycin (NB-DNJ), an inhibitor of ER-located α-glucosidases I and II, and or 1-deoxymannojirimycin (dMM), an inhibitor of the Golgi-located α-mannosidase I. The intracellular and secreted S1 proteins were analyzed by endoglycosidase H treatment. Similarly, ACE2 trafficking to the brush border membrane of intestinal Caco-2 cells was also assessed in the presence or absence of the inhibitors. Finally, the interaction between the S1 protein and ACE2 was investigated at the surface of Caco-2 cells by co-immunoprecipitation. Our data show that NB-DNJ significantly reduced the secretion of S1 proteins in COS-1 cells, while dMM affected S1 secretion to a lesser extent. Moreover, NB-DNJ and dMM differentially affected ACE2 trafficking and sorting to the brush border membrane of intestinal Caco-2 cells. Strikingly, the interaction between S1 and ACE2 was significantly reduced when both proteins were processed by the glycosylation inhibitors, rendering glycosylation and its inhibitors potential candidates for SARS-CoV-2 treatment.

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ASBMB

Abstract 2190

pH Effects on the Stability and Folding of Monomeric Caspases

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Clay Clark

Caspases are an ancient class of cysteinyl proteases that are integral in cell development and apoptosis as an evolutionarily conserved function. All apoptotic caspases evolved from a common ancestor into two distinct subfamilies: initiators, which are monomeric, and effectors, which are dimeric. Regulation of apoptosis is influenced by the activation mechanism of these two subfamilies, and how the subfamilies of monomers versus the dimers evolved from a well-conserved caspase-hemoglobinase fold is not well understood. We examined the folding landscape of monomeric caspases from two coral species, 300 million years distant on an evolutionary timescale between themselves and about 600 million years distant from human caspases. Since these coral caspases do not unfold via a distinct mechanism at all pH studied, the pH dependence on equilibrium unfolding is complex. Our results indicate that both proteins have overall high stability ~ 16 kcal mol-1 near the physiological pH range (pH 6 to pH 8) and unfold through a four-state mechanism via two intermediate states. The intermediate state (I2) is not sensitive to changes in pH, and the folding mechanism is mainly dependent on the stabilization of the native state and the intermediate (I1) state. Outside of this pH range, the native state is destabilized, whereas the intermediate (I1) is destabilized at extreme pH. Hence, the four-state model shifts to a three-state and a two-state as an effect of pH. We observe a decrease in the average emission wavelength of the native proteins and a decrease in the overall conformational stability below pH 6, which can be characterized by an estimated pKa ~ 5.7. This suggests a pH-dependent conformational change due to the protonation of a histidine residue, also observed in the dimeric family of caspases. Together, the data suggest that the folding landscape is conserved, with a possibly conserved allosteric mechanism. Furthermore, urea MD simulations data paired with limited proteolysis and MALDI-TOF indicate that the small subunit of monomeric caspases is unstable and could be the first to unfold, suggesting the importance of the evolution of the subfamily of stable dimers.

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Abstract 2193

Synthesis of the Transmembrane Domain of the Spike Protein from SARS-CoV-2 Using Solid Phase Peptide Synthesis and Determination of Its Oligomerization State

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Timothy Reichart

The spike protein in severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) is directly responsible for the binding to ACE2 receptors in host cells. While the spike protein overall is known to form trimers, the oligomerization state of the transmembrane domain of the spike protein in SARS-CoV-2 is unknown. It is believed to be essential for the function of this protein. Since the transmembrane domain of the spike protein is highly conserved in SARS-CoV-2 it is important to investigate its character and determine its relationship to the function of the protein as a whole. The goal of this project was to synthesize, characterize, and analyze the function of the transmembrane domain (TM) of the spike protein in SARS-CoV-2. The most practical method to synthesize the TM domain of the S protein is through solid phase peptide synthesis (SPPS). SPPS is a process in which peptides are made by linking amino acids, the monomers of proteins, one at a time until the full sequence is achieved. These peptide chains will then need to be purified using high-performance liquid chromatography (HPLC). The synthesized peptides will be analyzed using liquid chromatography-mass spectrometry (LCMS) to confirm the identity of the synthesized peptides as well as any potential impurities. The continued investigation of the S protein can lead to the discovery of small peptides capable of inhibiting key processes to the binding mechanism of SARS-CoV-2. The function of the S protein is believed to only present when the transmembrane domain forms a trimer. Therefore, the analysis of their oligomerization states will be investigated by synthesizing versions of the peptide that fluoresce when excited using dyes such as nitrobenzodiazole (NBD) and tetramethylrhodamine (TAMRA) in a fluorescence assay.

-Hampden-Sydney College Office of Undergraduate Research.

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Abstract 2196

Determining the Substrates of KDAC4 and KDAC7

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Lysine acetylation is a reversible post-translational modification process that occurs on cellular proteins. A specific group of enzymes, metal-dependent lysine deacetylases (KDACs), catalyze the removal of acetyl groups. This post-translational process has been widely observed, however, the overall function is not well understood. Putative biological substrates of the KDACs have been reported in the scientific literature, but few have been directly confirmed. In particular, the class IIa KDACs exhibit little catalytic activity *in vitro*, and there is little direct evidence of deacetylation by these enzymes. The goal of the project is to identify direct substrates of two class IIa enzymes, KDAC4 and KDAC7. To determine if KDAC4 and/or KDAC7 is/are responsible for the deacetylation of certain substrates, there are several methods being utilized. One is *in vitro* assays to determine if the enzymes are reacting with specific substrates by measuring the enzymatic activity present. Second, mammalian cell lines containing individually inactived KDACs are utilized as lysates and probed with specific antibodies for specific acetylated proteins. The antibodies react with putative substrates in the cell lysates which allows us to measure the amount of acetylation present. Third, cell lines with active or inactive KDAC4 and KDAC7 are used to isolate target proteins using immunoprecipitation and analyzed via western blots. Overall, our results directly show whether KDAC4 and/or KDAC7 are catalytically active with certain putative substrates. These findings are important as they provide a better understanding of the roles of KDAC4 and KDAC7 in cells.

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Abstract 2201

Overlapping biological functions and prion interactions of three yeast J-domain proteins

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Prions are self-propagating aggregates of misfolded protein that often form ordered amyloids. Amyloids are associated with a variety of human neurodegenerative diseases, including but not limited to Alzheimer's disease, Huntington's disease, and Parkinson's disease. Amyloid-forming prions are also present in *Saccharomyces cerevisiae*, where they are propagated through populations by a fragmentation mechanism that requires the chaperone proteins Hsp104, Hsp70 and the J-domain protein Sis1. When Hsp104 is ectopically overexpressed, however, the prion [PSI+] is specifically eliminated from cell populations in a Sis1-dependent manner. We previously determined that another J-domain protein Apj1 is required for the efficient elimination of strong variants of the [PSI+] prion by Hsp104 overexpression, and that the first 161 residues of Apj1 are sufficient for efficient prion elimination. We also previously determined that Apj1 and Sis1 have overlapping functions in the Hsp104-mediated curing and that Ydj1 can block curing when overexpressed. Here we further examined the portions of Apj1 necessary for efficient prion curing which led us to additional overlapping functionalities among these J-domain proteins. Various constructs produced by mutating and truncating regions of Apj1-161 were ectopically expressed in *Δapj1* cells to determine which regions, if any, were sufficient to replace Apj1 in Hsp104-mediated curing. Interestingly, when the J-domain alone of Ydj1 was overexpressed, it did not block curing, but was also sufficient to replace Apj1. Conversely, truncated constructs of Apj1 were more efficient than full-length Apj1 at rescuing the slow-growth phenotype of *Δydj1* cells. Finally, Sis1 is required for cell viability and cannot be replaced by the overexpression of any other full-length J-domain protein. Based on sequence alignments, we hypothesized that Apj1 may have more considerable functional overlap with Sis1 than previously appreciated. Shockingly, we found that C-terminal truncations of Apj1 can indeed replace Sis1 function and restore cell viability, revealing that Apj1 is in fact a pseudo-functional paralog of Sis1. This finding opened the door to ask if these Apj1 constructs can replace Sis1 in prion propagation as well; preliminary experiments indicate that this is the case for both [PSI+] and [RNQ+]. These results reveal a far more significant intertwinement of the structures and functions of these three J-domain proteins than has been previously appreciated and are significant in advancing our understanding of prion-chaperone interactions.

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Abstract 2211

Minimal phosphorylation sites required for SIKE dimer to monomer transition

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Structure dictates function and protein interactions mediate those functions. In the innate immune response to viral infection, Suppressor of IKK Epsilon (SIKE) is a protein of unknown structure and function, although several distinct protein interactions have been identified. Our long term goal is to understand how and why distinct SIKE interaction networks form and their impact on the innate immune response. We have defined SIKE as a primarily dimeric protein that undergoes a transition to a monomeric state when phosphorylated. We have also shown that SIKE phosphorylation modulates interactions with tubulin (enhances) and actinin (decreases). From our model of the SIKE dimer, five of SIKE's six phosphorylation sites per subunit are located at our predicted dimer interface suggesting that charge-charge repulsion of clustered phosphorylated serines mediates this quaternary state transition. Computational evaluation of these sites impact on dimer stability and their evolutionary conservation suggested that three of the five sites may be sufficient to induce this quaternary state transition. From these preliminary studies, we hypothesize that SIKE holds two distinct quaternary states that are regulated by phosphorylation at serines 187, 190, and 198. We have created a series of site-directed mutations representing individual and all combinations of these three serines mutated to glutamate. All bacterial expression of mutants was comparable to wild type levels. SEC of wild type SIKE shows multiple species, primarily dimer but also tetramer, which was confirmed by separating crosslinked WT-SIKE by SEC and assessing species present in peak fractions by SDS-PAGE followed by silver staining. Individual, double, and triple mutants were assessed in a similar manner. Results show how the introduction of negative charge redistributes the stable quaternary states and promotes the monomeric state.

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Abstract 2213

Arabidopsis β-amylase9 interacts with and enhances the activity of α-amylase3

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Starch accumulates in plastids to store excess sugar and is degraded when sugar is needed, at night or under stress, by a suite of enzymes including several β-amylases (BAM). Despite the obvious importance of starch in the human diet and for industry, we know very little about how starch degradation is regulated in plants. Of the nine BAM genes in the model plant *Arabidopsis thaliana*, BAM9 encodes a plastid-localized, catalytically-inactive pseudoenzyme suspected to play a regulatory role. In order to better understand the patterns of BAM9 expression we constructed transgenic plants expressing a BAM9-promoter:GUS gene. BAM9 appears to be expressed at tissue junctions, in guard cells, and in response to abiotic stress including wounding. Sequence analysis revealed two conserved surfaces of BAM9 so we carried out a yeast-2 hybrid search for BAM9-binding partners and isolated four overlapping partial clones from an α-amylase called AMY3. In addition to being located in plastids, AMY3 contains two N-terminal starch-binding domains (SBD) and a potential alpha-alpha hairpin region between the second SBD and the catalytic domain. Interestingly, each partial clone from the Y2H study contained the sequence associated with the putative alpha-alpha hairpin region. In order to explore this interaction, we purified His-tagged forms of AMY3 and BAM9 from *E. coli* cells, and found that BAM9 enhances AMY3 activity at least 2.4-fold. Mutants of BAM9 in the two conserved surface regions are being constructed to test the validity of this interaction. In addition, small-angle X-ray scattering (SAXS) was used to test the interaction between AMY3 and BAM9 using the programs ScÅtter and DAMMIF. Despite some degradation of AMY3, there was preliminary evidence of an interaction. We also confirmed that BAM9 is a monomer, and that it has an interesting N-terminal globular extension. Through these experiments we hope to gain insights into the role of BAM9 in regulating starch degradation.

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Abstract 2217

Parsing oxidation sensitivity and binding activity in the ribonuclease inhibitor protein

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Human ribonuclease inhibitor (RI) is a horseshoe-shaped leucine rich repeat (LRR) protein that inhibits pancreatic-type ribonucleases and acts as a sensor of cellular oxidative stress. RI is integral to a diverse array of biological activities, including translation initiation, stabilization of polysomes, maturation of miRNAs, regulation of miRNA target degradation, and inhibition of angiogenin-mediated activities such as rRNA synthesis and tRNA degradation. RI has a remarkably high cysteine content and is fully functional when its cysteines are reduced; oxidation of its cysteines results in its denaturation and inactivation. The long-term goal of this project is to dissociate the two key functions of RI: its oxidation sensitivity and its ability to inhibit ribonucleases. To accomplish this, RI variants were designed to examine the roles of specific cysteines in RI. Sets of cysteines, ranging from 5 to 32 residues, were replaced with leucines. Computational modeling of the variant RI proteins verified that the change in ΔG of folding was insignificant compared to WT. In addition to establishing novel protocols for the expression and purification of RI and its variants from mammalian cells, we are quantifying the effects of the cysteine substitutions on oxidation sensitivity, thermal stability, and binding to target ribonucleases. Preliminary results demonstrate that RI variants with up to 32 amino acid substitutions retain their ability to fold and bind to target ribonucleases. We will report on the Tm, KD for binding to RNase A, and the change in these biochemical constants under oxidizing conditions.

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Abstract 2223

Express and Purify Human CRY2 for Functional Studies

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Obed Lopez, Alberto Madariaga, Aleksander Lazarski, Chuan Xiao

Most cellular organisms have biological clocks known as circadian rhythms, which control the daily cycles of essential physiological and metabolic processes. At the cellular level, circadian rhythms are controlled by a time-delayed transcriptional-translational feed-back loop (TTFL) that involves many circadian proteins including cryptochrome (CRY). There are two paralogs of CRY within the human genome: CRY1 and CRY2, both having essential roles in the negative feedback of TTFL. The purpose of this project is to study the function of human CRY2 (hCRY2), especially its interaction with other circadian proteins. To reach this objective, it is essential to obtain pure hCRY2 by optimizing both expression and purification conditions of it. Advances of expression optimization, such as optical density of the culture, induction time, concentration of the inducer, and lysis methods have been accomplished through multiple trials. Through this research we would be able to express and purify large quantities of hCRY2 that will be used for future interaction studies. Disruptions of the circadian rhythm have been linked to numerous diseases, such as cancer, advanced sleep phase syndrome, insomnia, and jet lags, to name a few. The results from this project will deepen our understanding of the mechanisms behind the circadian rhythm and its associated diseases, facilitating the development of prevention and treatment strategies.

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Abstract 2229

Spectroscopic Analysis of Conformational Changes of Aconitase

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Virginia Smith

Human cytoplasmic aconitase is a large, multi-domain protein that functions as either a metalloenzyme or an RNA-binding protein, depending on intracellular iron levels. When the cell is replete with iron, the enzyme contains a [4Fe-4S] cluster in its active site that participates in isomerization of citrate to isocitrate. When iron levels fall, the cluster disassembles and the protein becomes human iron-responsive element binding protein-1 (hIRP1), which bind to mRNA elements involved in iron homeostasis. As part of the switch in functions, the protein undergoes a large conformational change that exposes previously buried surface area and creates a new binding site for the mRNA. Although the structures of the two functional, substrate or ligand-bound forms are known, we are interested in monitoring the changes that occur as the protein transitions between those forms. We have used fluorescence spectroscopy to characterize the environments of the intrinsic tryptophans, which are sensitive to quenching by the iron-sulfur cluster and polar solvent exposure. The use of near and far-UV circular dichroism allow us to monitor broader tertiary and secondary structural changes. Use of the extrinsic fluorophore ANS allows us to observe hydrophobic surface exposure during the transition. This research is supported by the Office of Naval Research Midshipman Research Fund and the United States Naval Academy Chemistry Department.

United States Naval Academy Chemistry Department Office of Naval Research.

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Abstract 2242

Genetic modulation of protein O-GlcNAcylation affects Atrial Fibrillation susceptibility

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Atrial fibrillation (AF) and diabetes mellitus (DM) are major, rapidly expanding public health problems. DM is a major independent risk factor for AF, but molecular mechanism(s) connecting DM to AF are unclear. Excessive O-GlcNAcylation (O-GlcNAc) is a hallmark of DM; however, the fundamental mechanisms by which O-GlcNAc promotes AF are not well understood. To test the role of O-GlcNAc in AF we developed transgenic (TG) mouse models with cardiac overexpression of the two enzymes that exclusively regulate protein O-GlcNAc – O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). In addition, we used both type 1 DM and type 2 DM mouse models. Both diabetic mouse models and non-diabetic OGT TG mice had increased myocardial O-GlcNAc and increased susceptibility to atrial burst pacing induced model of AF. Diabetic OGA TG mice were protected from increased AF susceptibility. Single dose treatment with an OGT inhibitor – 5-SGlcNHex at two different doses (50 mg/kg and 100 mg/kg) did not protect from increased AF in type 2 DM mice. These data support a role for O-GlcNAc as a mechanism for AF. Genetic O-GlcNAc inhibition but not pharmacologic O-GlcNAc inhibition at the doses and duration tested protected from AF susceptibility. Targeted O-GlcNAc inhibition is a potential therapeutic target for AF and DM, however further investigation is needed to translate this to clinical application.

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Abstract 2245

Characterizing the morphological differences in keratinocytes between a two-dimensional model versus a three-dimensional model

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Collagen is an extracellular matrix (ECM) protein that is involved in wound healing pathways. It contains a triple helix structure which forms collagen bundles at a neutral pH and is one of many extracellular matrices that can be used to create hydrogels. Gelatin is derived from collagen through hydrolysis. Due to gelatin's cross-linking properties, it is commonly used in food but also has applications in cell growth and has been commonly used to create three-dimensional hydrogels. The natural abundance of collagen present in gelatin allows for the optimization of cell growth and comparative analysis between environments of cells. Due to the mechanical differences in cell growth in the two-dimensional glass plate and the three-dimensional X-BlockTM (gelatin-based hydrogel), it can be hypothesized that there will be differences in the morphology and levels of proteomic expression between the two culture methods. To highlight the differences in keratinocyte morphology, stains commonly used in tissue histology will be utilized such as Hematoxylin & Eosin, Mason's Trichrome, Picrosirius Red, and Alcian Blue. With the utilization of Immunostaining and antibody tagging, Keratin 19, ITGB1, n-cadherin, and e-cadherin will be qualitatively analyzed to characterize keratinocyte morphology and proteomic localization. The colorimetric staining and immunostaining results will be expected to reveal cell morphology, proteomic expression, and protein localization that's similar to *in vivo* models. Additionally, by using Reverse Transcription PCR (RT-PCR) we will determine the expression levels of the 4 chosen proteins. It is expected to observe an increase in protein expression in the three-dimensional matrix versus the two-dimensional environment. This can be quantified through a western blot and analysis using the same 4 chosen proteins. These proteins have been selected because n-cadherin and e-cadherin have an inverse relationship in cellular growth, ITGB1 is well researched and from the migratory layer, and Keratin 19 is well documented and forms the basal layer. Using proteins from both the basal and migratory layers will give way for more specific research in the future.

This MAPS Team project is supported by the Center for Biomolecular Modeling and the Medical Professions Academy at Olathe North High School under the mentorship of Dr. AJ Mellott, Heather Decker, Dr. Aidyn Medina-Lopez, and Jacob Hodge, all of Ronawk Inc.

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ASBMB

Abstract 2269

X-Ray Crystallography of Zinc Solute Binding Protein ZrgA in *Vibrio cholerae*

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ZrgA is one of two extracellular solute binding proteins (SPB) believed to bind zinc in high affinity within the bacterium *Vibrio cholerae*. It is part of an ATP binding cassette transport system (ABC Transporters) which are responsible for the ATP powered translocation of different substrates across the membranes of almost all organisms from mammals to eubacteria. In an effort to combat the increasing rise of bacterial antibiotic resistance, these ABC transport systems have become attractive points of interest as they can possibly bring insight into new mechanisms in which we can control the virulence and pathogenicity of antibiotic resistant bacteria. *Vibrio cholerae* is the bacterium responsible for the acute infectious disease known as cholera and has emerged as a multidrug resistant enteric pathogen. ZnuABC is the first well- characterized ABC transporter of zinc observed within *Vibrio cholerae* however, the zrgABCDE transporter was recently identified to be another transporter of zinc. The ZrgA gene can be found in hundreds of organisms near other ABC transport genes, this implies its functions as an SBP. However, its independent function has not been characterized. This study aimed to focus on obtaining the unsolved structure of ZrgA through methods of X-ray crystallography. We observed several conditions of potential crystallization by running the protein through crystallization screening. A flexible region rich in His residues was observed on the ZrgA protein and believed to potentially interfere with crystallization. Therefore, a mutant sample with an approximate 60 residue knockout to the ZrgA protein was made. Protein was purified via column chromatography and the potential conditions were tested in abundance. Countless conditions were used to promote crystallization with only several conditions found suitable to achieve crystallization in the ZrgA protein. The crystal structure of both a zinc bound, and non-zinc bound ZrgA was obtained with high resolution and significant completeness. Thus, giving insight into the functionality and binding locations of the previously unsolved ZrgA protein.

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Abstract 2275

Interaction and Interplay between YAP1 and Polycomb protein SCML2 determines cell fate

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The evolutionarily conserved polycomb group protein SCML2 and yes-associated protein YAP1 regulate similar cellular biology, including gene expression, stem cell characteristics, and developmental processes in flies and mammals. However, the biochemical and functional association between SCML2 and YAP1 is unknown. Here, we identified SCML2 from the nuclear YAP1 protein complexes of human prostate cancer cell lines using immunoprecipitation and mass spectroscopy-based proteomics. Our cell fractionation, confocal microscopy imaging, and proximity ligation assay demonstrated that androgen hormone signaling regulates the SCML2 subcellular localization and interaction with YAP1. In addition, our GST-pulldown assays showed that SCML2 physically interacted with YAP1, and the WW/SH3 domain of YAP1 might mediate protein-protein interaction. Moreover, our siRNA knockdown studies revealed that SCML2 and YAP1 reciprocally regulate each other's activity. Furthermore, silencing SCML2 resulted in differential regulation of androgen-dependent and androgen-independent cell growth, likely due to a diverse modulation of the YAP and SCML2 protein-protein interaction by androgen signaling. Our findings suggest that the SCML2/YAP1 complex constitutes a novel molecular pathway for determining cell fate and contributing developmental disorders downstream of steroid hormones.

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Abstract 2276

eIF3 and its mRNA-entry-channel arm contribute to the recruitment of mRNAs with long 5′-UTRs

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Initiation is the most regulated phase of translation in eukaryotes. This multi-step pathway is mediated by numerous protein initiation factors (eIFs), of which eIF3 is the largest and most complex. eIF3 contributes to events throughout the initiation pathway, in particular playing critical roles in mRNA recruitment, a process which encompasses initial ribosome docking on an mRNA, scanning, and start-codon recognition. Recently, eIF3 has been implicated in driving the selective translation of specific classes of mRNAs in higher eukaryotes. Unraveling the mechanism of these diverse contributions — and disentangling the roles of the individual subunits of the eIF3 complex — nonetheless remains challenging. We have employed ribosome profiling of budding yeast cells expressing two distinct mutations targeting the eIF3 complex. These mutations either disrupt the entire complex or subunits positioned near the mRNA-entry channel of the ribosome. Recent Cryo-EM structures suggest that these mRNA-entry channel arm subunits relocate during or in response to mRNA binding and start-codon recognition. Our experiments show that disruption of either the entire eIF3 complex or specific targeting of its mRNA-entry-channel arm affects the translation of mRNAs with long 5′-UTRs and whose translation is more dependent on eIF4A, eIF4B, and Ded1 but less dependent on eIF4G, eIF4E, and PABP. Disruption of the entire eIF3 complex further affects mRNAs involved in mitochondrial processes and with more structured 5′-UTRs. We are now focused confirming these effects using reporter constructs possessing the 5′-UTRs of mRNAs most or least sensitive to each of these mutations, phenotypic assays investigating the sensitivity of eIF3 mutations to mitochondrial stressors, and *in vitro* biochemical assays aimed at elucidating the mechanistic origins of these effects and the collaboration between eIF3 and other PIC components. These latter experiments leverage a recombinantly reconstituted eIF3 complex that enables its full dissection within an *in vitro* biochemical system which we are developing in collaboration with the laboratory of Ruben Gonzalez.

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Abstract 2279

In vitro formation of cataract as a model for age-related cataract

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Age-related cataract affects 22% of individuals over the age of 75 years and data suggest that delaying progression rate by 10 years would decrease cataract surgery by 50%. When rodent lenses are incubated in culture medium, they lose their transparency after 24–48 hours because protein aggregation changes the index of refraction resulting in light scattering and opacity. We hypothesized that finding ways to prevent or delay this process through manipulation of the culture medium would provide important clues toward cataract prevention and present a solid foundation for drug testing model on specific mutated mice lens for cataract treatment. 13 culture conditions each comprising 4–6 adult C57BL6 mice lenses were tested whereby variations in glucose, pyruvate, fructose, antioxidant, osmolality (sorbitol), crowding agent (polyethylene glycol) were varied. The extent of opacification, the type of opacity (nuclear vs cortical) and the lens diameter were determined as of function of incubation time. Results show that compared to the baseline conditions (Medium TC199), basic nutrition materials that lenses need for survival and antioxidants that prevents oxidative stress in lens show no improvement in preventing lens opacification. Next, we mimic the lens environment in the eye and test hyperosmolarity. PEG 1000 stood out among the rest by successfully preventing cataract formation in all of its lens. We also notice the lens size difference between PEG 1000 condition and the rest. PEG 1000 successfully prevents the lens from swelling and keep lens volume low. Thus, from our result, we tested the another set of biopolymers which could prevent massive intake of medium to the lens that we saw in previous conditions. We saw that Lymphoprep successfully kept lens clear until day 4 (96 hours), which was a major improvement compared to the rest of the conditions that we tested. In conclusion, this project would be novel in approach as the first to develop a system of drug testing lens model for cataract by keep mice lenses clear and transparent for 7–14 days, while we were still on the process of reaching 7–14 days of clarity in lens, we already tested out some strong potential conditions that improved lens transparency *in vitro* incubations such as PEG 1000 & Lymphoprep. We would further test on the possible conditions for optimizing lens culture medium for *in vitro* lens transparency. Specifically, we plan to focus on mimicking lens environment in eye to pertain lens homeostasis.

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Abstract 2280

Investigating the Interaction Between BECN1 and TAB2

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Autophagy is a cellular degradation pathway responsible for recycling damaged and malfunctioning proteins and organelles. Dysfunctional autophagy, which leads to excess recycling or an accumulation of damaged or harmful cell content, has been linked to cancer tumorigenesis and neurodegenerative diseases. BECN1 is a core protein that participates in and increases the activity of the VPS34 complex, leading to autophagosome nucleation. Transforming growth factor B-activated kinase 1 (TAK1) binding protein 2 (TAB2) has dual functions in autophagy, serving both pro- and anti-autophagy roles. During normal cellular conditions, TAB2 interacts with cytoplasmic BECN1 inhibiting autophagy. TAB2 dissociates from BECN1 during the induction of autophagy, promoting autophagy through both, the release of BECN1 allowing it to participate in the VPS34 complex, and the release of TAB2 which binds to and activates TAK1, activating the IKK signaling pathway.1, 2 The goal of our research is to understand the mechanism of interaction between BECN1 and TAB2 using a range of structural, biophysical, and biochemical methods. Previously, yeast two-hybrid assays and coimmunoprecipitation experiments have shown that the coiled coil domain (CCD) of both proteins are necessary for the interaction.2 Our circular dichroism data indicates that the TAB2 CCD becomes more helical when in complex with BECN1 CCD. Microscale thermophoresis (MST) shows that the TAB2 CCD binds to the BECN1 CCD with an affinity that is 4-fold and 70-fold tighter than the homodimerization of either the BECN1 CCD or the TAB2 CCD respectively. Unfortunately, our attempts to crystallize the BECN1:TAB2 CCD heterodimer have so far been unsuccessful, therefore, we used the multimer function in the AlphaFold 2.0 to predict the heterodimer structure and identify potential interface residues.3 We have mutated these interface residues and are using MST to quantify the impact of these mutations on BECN1:TAB2 heterodimerization. These results will provide insight into the packing interface between the BECN1:TAB2 CCDs. (1) A, C.; L, S.; H, A.; MC, M.; E, M.; I, V.; O, K.; E, T.; L, G.; S, S.; et al. The IKK Complex Contributes to the Induction of Autophagy. The EMBO journal 2010, 29 (3). DOI: 10.1038/emboj.2009.364. (2) A, C.; M, N.-S.; SA, M.; M, M.; E, M.; G, M.; S, L.; AV, A.; F, H.; G, P.; et al. Inhibition of Autophagy by TAB2 and TAB3. The EMBO journal 2011, 30 (24). DOI: 10.1038/emboj.2011.413. (3) Evans, R.; O'Neill, M.; Pritzel, A.; Antropova, N.; Senior, A.; Green, T.; Žídek, A.; Bates, R.; Blackwell, S.; Yim, J.; et al. Protein complex prediction with AlphaFold-Multimer. 2021. DOI: 10.1101/2021.10.04.463034.

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Abstract 2283

Using a Molecular Docking Approach to Evaluate Potential Neurosteroid Ligands for StarD6

Gabriella Papale, *Salve Regina University*

Alzheimer's disease (AD) is a degenerative brain disorder and the main cause of dementia, a syndrome characterized by defects in memory, language, problem solving, and other cognitive abilities. While it is well understood that accumulation of beta-amyloid (Aβ) plaques and abnormal tau protein ("tau tangles") are associated with the neurological degeneration of AD, the molecular mechanisms that occur early in the progression of the disease remain somewhat elusive. It was recently reported that a single nucleotide polymorphism (SNP) in the promoter region of the gene encoding StarD6 may be correlated with AD risk. StarD6 is expressed in testis, ovary, and in brain regions such as the hippocampal formation, the degeneration of which is implicated in the progression of AD. StarD6 binds cholesterol, testosterone, and pregnenolone, and it has been proposed that this protein also may also bind cholesterol-derived neuroprotective steroids. Furthermore, expression of this protein increases in the hippocampus with age and after excitotoxic brain injury. Previous studies using nuclear magnetic resonance (NMR) and molecular dynamics (MD) simulations have indicated several amino acid residues in binding testosterone and cholesterol, and it is likely that both ligands associate with these residues through the formation of multiple hydrogen bonds. However, there is currently no published structure available for the ligand-bound protein, so further investigation is required to fully characterize the bonding interactions between these ligands and StarD6. Due to the shape and volume of its binding cavity, it likely binds related compounds as well (including neurosteroids). The purpose of this study was to validate the formation of hydrogen bonds between amino acid residues in the StarD6 binding cavity and its ligands, both known and putative. We hypothesize that the inherent redundancy of amino acid residues capable of hydrogen-bonding with ligand molecules in the StarD6 binding cavity result in a heterogeneous population of StarD6 ligands. We used Molecular Operating Environment (MOE), a molecular design platform from Chemical Computing Group, to perform docking studies using the published solution structure of StarD6 (PDB ID: 2MOU) and several known and putative StarD6 ligands. Docking was performed using four different placement methods: Alpha PMI, Alpha Triangle, Proxy Triangle, and Triangle Matcher. Each placement method was further refined using either Rigid Receptor or Induced Fit, and both the placement and refinement methods were scored using multiple methods: GBVI/WSA dG, London dG, Alpha HB, Affinity dG, and ASE. We determined the optimal combination of placement, refinement, and scoring methods to be Proxy Triangle (placement), Induced Fit (refinement), and GBVI/WSA dG (score), as the calculated S-values for testosterone and cholesterol (−7.3130 and −7.0162, respectively) best mirrored their published Kd values. Through this method, we identified four putative ligands with a lower S-value than testosterone: androstanediol, DHEA, estradiol, and etiocholanolone; a lower S-value suggests that these neurosteroids have a higher affinity for StarD6. Four residues were identified as participants in hydrogen bond formation: L65, R71, C151, and N198. We are currently focusing on R71, C151, and N198 for future mutagenic docking studies in which these residues will be substituted with residues incapable of forming hydrogen bonds.

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Abstract 2291

Determining where the protective protein, CowN, binds to nitrogenase

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Dustin Willard, Cedric Owens

Nitrogenase is the only enzyme that can reduce nitrogen gas (N2) into the plant nutrient ammonium (NH3). Carbon monoxide inhibits nitrogenase by binding to its active site and preventing NH3 production. The protein CowN interacts with the Molybdenum-Iron Protein (MoFeP) component of nitrogenase. The interaction weakens CO's inhibition constant, thereby protecting nitrogenase from CO. The aim of this work is to determine how CowN and MoFeP interact through mutational analysis and protein crosslinking. We hypothesized that CowN binds MoFeP using its C-terminus and that CowN binds MoFeP either near its active site and/or at the entrance of a gas channel. Mutations were made on the C-terminus of CowN. Mutations at glutamate 87 (E87) prevent crosslinking, which indicates that E87 is necessary for protein-protein interactions to occur. Mutations at other C-terminal sites did not abolish crosslinking, suggesting E87 has a key role in establishing the interaction with MoFeP. To detect where on MoFeP's surface CowN binds, crosslinking was conducted with SIAB, which crosslinks sulfhydryl groups withs primary amines. These experiments were conducted with both wild-type CowN and a CowN mutant that did not have any cysteine residues. Both wild-type and the no-cysteine mutant crosslinked equally well. This indicates that the sulfhydryl-reactive group of the crosslinker binds to MoFeP and thus that CowN binds to a site on MoFeP that features a cysteine. These results narrow down CowN binding to three cysteine-containing surface sites on MoFeP. The aim of future work will be to identify which of the three sites is the target for CowN binding.

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Abstract 2304

Using differential scanning fluorimetry to evaluate the significance of hydrogen bonding between StarD6 and its ligands

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Caitlin Bessette, Kira Spedden, Gabriella Papale

Alzheimer's Disease (AD) is a neurodegenerative disease that is the leading cause of dementia. It is characterized by decreased cognitive function, loss of memory, and the formation of beta-amyloid plaques and tau tangles in the brain. Interestingly, the biochemical mechanisms that initiate the progression toward AD remain elusive. Recently, studies have found that a single nucleotide polymorphism (SNP) in the promoter region of the gene for StAR related lipid transfer domain containing 6 (StarD6) may be associated with the development of AD. StarD6 is a protein that is expressed in the brain and testes and binds steroids such as testosterone and cholesterol. Previous NMR spectroscopy studies and molecular dynamics simulations have revealed several key residues that may be involved in binding testosterone: arginine 71 (R71), lysine 76 (K76), tyrosine 81 (Y81), glutamine 99 (Q99), serine 100 (S100), aspartate 113 (D113), serine 129 (S129), lysine 186 (K186), and asparagine 191 (N191). Due to the chemical nature of the residue side chains, testosterone likely associates with these residues through the formation of hydrogen bonds. We hypothesize that due to the number of hydrogen bonding groups present in the StarD6 binding cavity, some amino acid residues may be absolute requirements for ligand binding, while others may be redundant. To date, no additional studies have been completed to validate these residues and further characterize the ligand binding site of StarD6. To that end, we used site-directed mutagenesis to create two sets of StarD6 mutations: one set that eliminates H-bonding and one set that preserves H-bonding. In the mutation set that eliminates H-bonding, amino acid residues that can form hydrogen bonds were substituted with amino acids of a similar size and shape that are unable to form hydrogen bonds, such as substitution of aspartate with alanine; this panel of mutations consists of R71L, K76M, Y81F, Q99L, S100A, D113A, S129A, K186M, and N191V. In the mutation set that maintains H-bonding, the original amino acid residues were instead substituted with residues that maintained the ability to form hydrogen bonds, such as substitution of aspartate with glutamate; this panel of mutations consists of R71K, K76R, Y81H, Q99N, S100T, D113E, S129T, K186R, and N191Q. We have successfully generated and purified six of the 18 total mutants: R71L, R71K, K76M, K76R, Y81F, and Y81H. In order to test the ability of these mutants to bind cholesterol, we are utilizing a fluorescence-based isothermal binding assay (differential scanning fluorimetry, or DSF). DSF is a modified thermal shift assay that allows for the quantitative determination of a ligand's binding affinity at a single temperature near the protein's melting temperature (Tm) using varying ligand concentrations. After testing several potential buffers, we have

determined that the optimal buffer for StarD6 in this assay is 120 mM NaCl, 20 mM NaH2PO4/Na2HPO4; we have also optimized this assay for concentrations of both StarD6 protein and one of its known ligands, cholesterol. We are currently using the optimized DSF assay to test cholesterol binding to both wild-type and mutant StarD6, as well as to evaluate potential ligands for StarD6, such as the neurosteroids allopregnanolone and DHEA.

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Abstract 2305

In Vitro Dissection of eIF3 and its Role in mRNA Recruitment Using a Recombinantly Reconstituted Complex

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Amber Huang, Nick Ide, Colin Echeverría Aitken

Translation initiation is the most highly regulated step of translation. Eukaryotic translation initiation factor 3 (eIF3) is integral to the process of translation initiation, and altered expression of each of the five core functional subunits of the complex has been linked to the proliferation of a number of cancers. However, studying mutations in the 5 essential subunits of eIF3 in *S. cerevisiae*, a model eukaryote, is difficult because mutations that severely damage the complex lead to yeast cell death and prevent purification of the mutated eIF3 complex. To circumvent this issue, we are reconstituting eIF3 with subunits recombinantly expressed in *E. coli*, and we are performing binding experiments and ribosome profiling to determine how various mutations and conditions affect translation initiation. This allows us to express mutated variants of eIF3 and track the effects of mutations on translational efficiency, specifically the effects of these mutations on pre-initiation complex (PIC) formation, binding of eIF3 to the ribosome, and mRNA recruitment. We make use of a reconstituted system comprised of initiation factors, RNAs, ribosomes, and other molecules necessary to initiate translation and perform binding experiments that track mRNA recruitment to the PIC and the effects of eIF3 on this process. We are now exploring interactions between wild-type eIF3 and a 40S subunit variant that contains a single amino acid mutation in a latch protein at the the mRNA entry channel, where eIF3, the ribosome, and mRNA all interact to begin the process of translation. We have observed decreased binding between eIF3 and the ribosome as a result of this mutation. We are now working towards testing other mutations in the binding region and the effects of various mutations in eIF3 on binding efficiency between eIF3 and the ribosome to shed light on the function of specific subunits of eIF3 and how specific mutations in certain regions are linked to changes in translation initiation.

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Abstract 2310

Structural and functional support to extend the fumarase C active site landscape

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Gage Stuttgen, Kelly Gorres, John May, Basudeb Bhattacharyya

Fumarase, a Krebs Cycle enzyme, catalyzes the reversible conversion of fumarate to S-malate. The functional form of fumarase requires oligomerization into a homotetramer. Fundamentally, each of the four fumarase active sites are constructed from three regions of amino acids that share identity amongst family members. These three regions, spatially distant within the three-dimensional monomeric structure, coalesce upon homotetramerization to form four concave active sites. An auxiliary carboxylate binding site, built from R126 and a small segment of π-helix, has been discovered previously. This site, termed the B-site, is located ~15 Å from the active site and its role within fumarase family has not been fully defined. We substituted arginine 126 for an alanine to construct the R126A variant. We purified R126A and conducted steady-state kinetics, circular dichroism and thermofluor stability studies. Additionally, X-ray crystallography was used to solve the R126A structure to 1.36 Å resolution. Analysis of the steady-state kinetic results demonstrated that R126A had elevated Km and Ki for (meso-tartrate) values and led to a lowered turnover number. Structurally, substitution of R126 with an alanine diminished meso-tartrate induced thermal stability and eliminated the crystallographic observation of citrate at the B-site. Crystallographically resolved water molecules now occupy the original position of the R126 side chain guanidinium functional group. In conjunction, an ion pair between R126 and E7, within the N-terminal domain, is lost. H129 and N135 also shift conformations within the B-site. We conclude the B-site, facilitated by the positioning of R126, facilitates substrate binding within the fumarase active site.

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Abstract 2316

Antifreeze Protein Type I and its Use in Cryopreservation

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Tejpreet Kaur, Kathryn Bruss

Antifreeze proteins (AFP) are a class of proteins that inhibit the formation of ice crystals internally in organisms that have adapted to live in dangerously cold environments. These proteins can be found in bacteria, fungi, crustaceans, microalgae, insects, and fish. There are two different ways an AFP can prevent cell damage due to ice, thermal hysteresis (TH) and ice recrystallization inhibition (IRI). More often insect AFPs have a higher TH activity while fish have a higher IRI activity. AFPs with high TH activity are used for static cold storage at subzero temperatures, while AFPs with high IRI activity help enable cryopreservation. The most suitable method for the long-term storage of single cells in ultra-low temperatures is cryopreservation. In polar fish AFPs have diverged into three main types from convergent evolution, these types each have different structures performing similar functions. The focus of this study was on AFP type I, small proteins that are most commonly found in winter flounder. These fish live in the Northwest Atlantic continental shelf in water temperatures ranging from 28 to 50°F. Winter Flounder reproduce in estuaries throughout the shelf during the winter. The species' ability to tolerate the cold water temperatures allows the fish to reproduce where few predators are able to survive, giving their spawn an evolutionary advantage. The AFP type I protein consists of an alanine-rich alpha-helix, with four threonine hydroxyls, which are spaced equally apart on one face of the helix. The protein is mainly used in the cryopreservation of sperm cells as it is able to increase the protection of the cells while undergoing cryopreservation during sperm banking. Without an AFP the sperm cells may undergo osmotic shock and membrane damage, ultimately leading to cell death when thawed.

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Abstract 2319

High-throughput Identification of Calcium Regulated Proteins Across Diverse Proteomes

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Rose Fields, Hayden Gizinski, Melissa MacEwen, Devin Schweppe, Yasemin Sancak

Mitochondrial calcium plays a well-known regulatory role in mitochondria. Disruption of mitochondrial Ca2+ uptake is implicated in diseases such as cancer, neurodegenerative, and metabolic diseases. Evaluating the relationship between the disease states and mitochondrial Ca2+ uptake has proven difficult due to limited knowledge of the mediators of mitochondrial Ca2+ signaling. Currently, there are 18 known mitochondrial Ca2+-binding proteins, which were identified using targeted biochemical assays or computational detection of EF-hand domains. The existence of other calcium-regulated mitochondrial proteins with non-canonical Ca2+-binding domains, which are resistant to computational detection, is unknown. We set out to identify novel mitochondrial Ca2+-binding proteins in a high-throughput and unbiased manner and investigate how Ca2+ ions regulate these proteins and the mitochondrial pathways they control. To identify calcium-regulated proteins, we optimized a biochemical assay, PISA, that detects the conformational changes in proteins after they interact with calcium. We performed PISA on multiple samples - human cells, yeast cells, and mouse mitochondrial enriched samples from liver tissue– and showed the cross-species viability of this assay to find calcium-regulated proteins. Focusing on the mitochondrial samples, we correctly identify known Ca2+-binding and non-Ca2+-binding proteins in an unbiased manner, as well as covering above 85% of the mouse liver mitochondrial proteome. Towards understanding the calcium-regulation of select hits, we have used microscale thermophoresis (MST) to detect calcium-binding *in vitro* at physiologically relevant free calcium concentrations, successfully identifying novel mitochondrial calcium-binding proteins. Our results fill a large hole in the field's knowledge of mitochondrial Ca2+ signaling and provide multiple avenues for further research by highlighting new molecular players through which mitochondrial Ca2+ regulates mitochondrial functions.

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Abstract 2329

Investigating the contribution of distinct regions of the eIF3 complex to mRNA recruitment

Amber Huang, *Vassar College*

Lucy Funes, Shanya Galbokke Hewage, Christine Xu, Sophie Anderson, Colin Echeverría Aitken

Translation initiation is the most regulated and the rate-limiting step of translation. During initiation, mRNA must be recruited to and positioned on the ribosome, which must scan for the start codon to begin translation. Eukaryotic initiation factor 3 (eIF3), the largest of the eukaryotic initiation factors, is implicated in many steps of translation initiation, including mRNA recruitment and start-codon recognition. However, the mechanisms of how eIF3 and its mRNA entry-channel arm recruit and scan mRNAs remain unknown. Previous ribosome profiling work in our lab has identified mRNAs whose translation is sensitive to mutations in the mRNA entry channel (mEnC) arm of eIF3 or degradation of the entire eIF3 complex. We are employing an *in vitro* assay that reconstitutes translation initiation *in vitro* with eIFs, ribosomal subunits, tRNA, and mRNAs sensitive to eIF3 mutations to investigate the kinetics and recruitment efficiencies of sensitive mRNAs with wild-type and mutant eIF3. By identifying characteristics in the 5′ untranslated regions of these mRNAs that make them sensitive to mutations in eIF3, we will illuminate the molecular mechanisms by which eIF3 and its component subunits assist in mRNA recruitment. We have established a library of expression plasmids for *in vitro* transcription of sensitive mRNAs for analysis in our reconstituted assay. We are also exploring the translational effects of mutations in eIF3 through several whole-cell approaches including luciferase reporter assays, RT-qPCR of fractionated translational extracts, and tandem mass tag-mass spectroscopy. Together, these approaches will illuminate the mechanisms by which eIF3 participates in translation initiation.

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Abstract 2330

The Role of Ubiquitin Activating Enzyme (E1) Uba1's Ubiquitin Fold Domain on Ubiquitin Conjugating Enzyme (E2) Selection

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Ian Fucci, R. Andrew Byrd

Ubiquitin is a small, stable, and highly conserved protein expressed in eukaryotes. As a posttranslational modification, ubiquitin can be covalently attached to protein substrates in a variety of states: as a single unit, and as homo-polymeric or hetero-polymeric chains, resulting in a variety of downstream effects. The nature of the modification codes for biological processes such as degradation by the proteasome and altered localization or protein function. This covalent modification, called ubiquitination, occurs by a cascade of three enzymes: an E1 activating enzyme, an E2 conjugating enzyme, and an E3 ligating enzyme. During the series of enzymatic reactions, E1 loads the E2 with ubiquitin in an ATP-dependent fashion, the E2 transfers ubiquitin to the E3 and dictates the ubiquitin polymeric chain type, and the E3 transfers ubiquitin to the substrate. In the human genome, the differing abundance of these enzymes reflects this proposed function, with 2 known E1s, approximately 40 E2s, and more than 600 E3s. Progress toward understanding the mechanisms of recognition between E1 and E2 will be presented. The ubiquitin fold domain (UFD) of the E1 Uba1, which is a domain responsible for E2 selection, and the E2 conjugating enzyme UbcH5B, were expressed and purified in *E. coli*. Various fusion constructs were explored to aid solubility and expression of the UFD, wherein a cleavage site between the fusion partner and UFD enabled post-purification cleavage *in vitro*. Titrations will be performed between the UFD and UbcH5B to reveal NMR chemical shift perturbations to identify residues on each side of the interface, thus revealing the E1-E2 interaction. Site-directed mutagenesis experiments will confirm the interactions and provide additional structural information about the binding interface. Subsequently, additional E2 enzymes will be expressed, and NMR experiments will explore the specificity of the E1 UFD-E2 interactions.

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Abstract 2333

Binding profiles of BRAF reveal the roles of regulatory domains in auto-inhibition and the RAS-RAF interaction

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Zhihong Wang

The serine threonine kinase, BRAF, is a key member of the MAPK signaling pathway which directs normal cell growth, reproduction, and differentiation; however, mutated or mis-regulated BRAF is the source of numerous cancer types. Additionally, BRAF is one essential effector of RAS and is involved in promoting various cancers when RAS contains activating mutations. The RAS-RAF interaction is nuanced and is closely related to the complex activation of BRAF. Through RAS interaction and membrane recruitment, BRAF switches from an inactive, auto-inhibited monomer to an active dimer. Most current evidence surrounding the regulation of BRAF comes from static structures, cell-based assays, or computational modeling, but does not include extensive *in vitro* binding data. We proposed that studying the inter- and intra-molecular interactions of BRAF with itself and RAS would unveil domain-specific and isoform-specific details of RAF activation and auto-inhibition. Through pulldowns, surface plasmon resonance (SPR), and hydrogen-deuterium mass spectrometry (HDX-MS), we investigated the binding between BRAF N-terminal regulatory and kinase domains, as well as the binding between the N-terminal regulatory domains of BRAF and RAS. The binding data provide direct evidence regarding the relief of BRAF auto-inhibitory interactions by RAS binding and highlight the individual roles of the BRAF regulatory domains, including the BRAF specific region, RAS binding domain, and cysteine rich domain. The different binding profiles between HRAS and KRAS towards BRAF suggest an isoform-specific activation mechanism of BRAF under physiological conditions. The insights shown here are useful for developing new strategies toward therapeutic intervention, which are much needed for the enigmatic and challenging BRAF kinase.

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Abstract 2355

Biomolecular condensates in regulating cGAS-STING-mediated antitumor immunity

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Cytosolic DNA sensing by cGAS-STING signaling pivotally controls infectious, autoimmune, malignant, and degenerative diseases. However, how these innate immune responses are precisely controlled by specific cellular compartmentation, such as biomolecular condensation, is less understood. On the other hand, missense mutations of tumor suppressors, including Neurofibromin 2 (NF2/Merlin/schwannomin), often result in sporadic to frequent occurrences of tumorigenesis in multiple organs, although the underlying pathogenicity remains incompletely known. We found that, although NF2 functioned positively to innate immunity via regulating YAP/TAZ-mediated TBK1 inhibition, patient-derived individual mutations in the FERM domain of NF2 (NF2m) converted NF2 into a potent suppressor of cGAS-STING signaling. Mechanistically, NF2m gains extreme associations to IRF3 and TBK1 and, upon DNA sensing, is directly induced by the activated IRF3 to form cellular condensates, sequestering TBK1 and eliminating its activation by the PP2 complex. Accordingly, NF2m robustly suppressed the STING-initiated antitumor immunity in cancer cell-autonomous and nonautonomous murine models, and NF2m-IRF3 condensates were evident in human vestibular schwannomas. Other observations in disease-causing oncogene mutants proposed more complex and elaborate regulation of cGAS-STING signaling. Therefore, we will discuss our intriguing findings regarding phase separation-mediated quiescence and activation of cGAS-STING signaling and how these gain-of-functions by disease-causing mutations into antitumor immunity and pathogenesis.

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Abstract 2362

High-resolution Crystal Structures of Transient Intermediates in the Phytochrome Photocycle

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Phytochromes are red/far-red light photoreceptor enzymes in bacteria to plants, which elicit a variety of important physiological responses. They display a reversible photocycle between the resting (dark) Pr state and the light-activated Pfr state. The light signals are transduced as structural change through the entire protein to modulate the enzymatic activity. It is unknown how the Pr-to-Pfr interconversion occurs as the structure of intermediates remain elusive. Here, we present crystal structures of the bacteriophytochrome from myxobacterium *Stigmatella aurantiaca* captured by two different X-ray Free Electron Lasers, the Spring-8 Angstrom Compact free electron LAser (SACLA) and the Linac Coherent Light Source (LCLS). The structures were determined at early time points after light illumination of the Pr state. We observe large structural displacements of the covalently bound bilin chromophore, which trigger a bifurcated signaling pathway. The snapshots show with atomic precision how the signal progresses from the chromophore towards the output domains, explaining how plants, bacteria and fungi sense red light.

The research was funded by NSF-STC 'Biology with XFELs (BioXFEL)', award number 1231306. E.A.S. is supported by NSF-MCB-EAGER 1839513 and NSF STC BioXFEL center sub-award 6227. L.A. and M.N. are supported by NIH T34GM 105549.

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ASBMB

Abstract 2365

Quantifying glycoprotein mixtures using differential scanning calorimetry

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Milk serum contains valuable glycoproteins. Due to the individual benefits, there is an increasing interest in quantifying each glycoprotein amount in milk. However, quantities of these milk proteins vary depending on the breed, feed, and the health of cows. Using differential scanning calorimetry (DSC), the enthalpy and unfolding temperature (Tmax) can be found for each glycoprotein and be used to quantify the amount in each milk sample. When running a sample of milk serum on the DSC, the peaks of the various glycoproteins overlap. The overlapping makes it difficult to quantify all the glycoproteins in one thermogram. Protein unfolding can be influenced by different pH values and binding ligands. Two of the glycoproteins, α-lactalbumin and lactoferrin, have binding affinity for calcium and iron, respectively. In this study, we characterize how pH and binding ligands change the enthalpy and Tmax of each protein individually, thereby making it possible to quantify the mixture of glycoproteins in one thermogram. We have determined that the Tmax for apo lactoferrin (iron free) shifted from 51.28 ± 0.29 to 62.51 ± 1.55 to 64.06 ± 0.15 at pH 4.8, 7.8, and 10.2 respectively. The holo lactoferrin (iron bound) Tmax shifted 76.51 ± 0.23 to 87.28 ± 0.55 to 82.93 ± 0.57 at pH 4.8, 7.8 and 10.2. The lactoperoxidase Tmax 60.33, 67.77, and 72.47 at a pH of 4.8, 7.8, and 10.2. We also see shifts in the profile of the milk serum at different pHs. Milk serum proteins are thermal-sensitive to pH and mineral concentrations that can be used for protein quantification.

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Abstract 2376

The potential role of novel Small Protein 21 interacting with methyl-accepting chemotaxis proteins in *P. aeruginosa*

Steven Yu, *Amherst College*

Samuel Grondin, Zachary Jonas, Mona Orr

Pseudomonas aeruginosa is a Gram-negative, rod-shaped, nosocomial opportunistic human pathogen that is propelled via swimming motility by a single polar flagellum or via twitching motility by pili. *P. aeruginosa* senses and moves up gradients of attractants such as nutrients and down gradients of repellents such as toxic compounds. This is known as chemotaxis, the movement toward or away from a chemical attractant or repellent. Chemotaxis is controlled by a complex chemosensory system and composed of numerous chemosensory proteins. This is a well-studied system in *P. aeruginosa*, however, novel components of this system may yet be discovered. Preliminary data from this lab has identified a 43-amino acid long small protein that co-purifies with the methyl-accepting chemotaxis protein PctA, which senses amino acids. Evidence of this protein was initially provided via ribosome profiling and expression was confirmed in the lab via epitope tagging and immunoblotting. The objective of this work is to characterize the relationship between Small Protein 21 and PctA. Using recombineering techniques, we created a gene knockout, ΔsmORF21, and assayed the viability of this knockout under different conditions and probe for effects on chemotaxis. This small protein was induced upon membrane stress induced by SDS and EDTA, and we observe that ΔsmORF21 exhibited reduced growth compared to wild-type in membrane stress. There also appears to be a chemotaxis defect on casamino acid soft agar plate-based swim assays that include SDS and EDTA. However, the reduced swim radius observed in ΔsmORF21 compared to wild type cannot be solely attributed to a chemotaxis defect due to the observed growth defect. Thus, single-cell microscopy assays are being developed to examine the effect the deletion of this small protein has on chemotaxis. In conclusion, our results indicate that Small Protein 21 may be important in chemotaxis under membrane stress conditions and a better understanding of its function may provide critical insight into bacterial chemotaxis and the response to bacterial envelope stress.

This research has been supported by the Amherst College Biology Department.

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Abstract 2383

Environmental Stresses Induce Distinct SUMOylation Responses

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Environmental stresses disrupt homeostasis by altering cellular processes and damaging molecular components. To survive such conditions, cells must rapidly sense the stress and initiate the appropriate adaptive responses. These processes are often dependent on protein post-translational modifications. Here, we demonstrate that multiple environmental stress conditions elicit distinct small ubiquitin-like modifier (SUMO) responses. Additionally, protein SUMOylation patterns correlate to the intensity and duration of the stress. The majority of stress-induced SUMO substrates detected by mass spectrometry are transcription factors and other chromatin-binding proteins. This suggests that SUMO plays a role in regulating chromatin stability and gene expression during acute cellular stresses. The goals of our research are to characterize the diversity of SUMO stress responses across conditions and species and to better elucidate the signaling pathways involved in restoring cellular homeostasis.

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Abstract 2385

Exploring Protein Interactions of a Secondary Messenger Pathway Within Streptomyces

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Evan Shelton, Jennifer Bennett

The species *Streptomyces coelicolor* is a member of a genus that is particularly interesting for antibiotic research. *S. coelicolor*, when exposed to stimuli, enacts the use of a second messenger, called cyclic di-GMP, which signals for the natural production of antibiotics, biofilm formation, life cycle progression, and several other major functions within the cell. It is believed that the signaling within bacteria is largely dependent on the local concentration of cyclic di-GMP. Effective elimination of phosphodiesterases and diguanylate cyclases has been observed to greatly affect the cells of *E. coli* without changes in the level of cyclic di-GMP present. The major purpose of this research is to isolate and test for interactions between the two major cyclic di-GMP phosphodiesterases (RmdA and RmdB) of *S. coelicolor*. Addressing the biochemistry focus of this project, these proteins have been overexpressed within *E. coli*. From there, purification of individual proteins will allow for the testing of interactions among RmdA with itself, RmdB with itself, and between the two using Surface Plasmon Resonance (SPR). The other avenue of this investigation involves a Bacterial Two-Hybrid (BACTH) system, to further confirm these findings and serve as a more visual representation of protein interaction. Preliminary SPR experiments show an interaction between RmdB and itself, suggesting homodimer formation is important for protein function. Additionally, recent preliminary BACTH experiments suggest RmdA will also form a homodimer for interaction. The next steps of experimentation will aim to determine whether the formation of a heterodimer occurs as part of this secondary messenger pathway. There is still much to understand about the functioning of the cyclic di-GMP pathway in Streptomyces. This project seeks to discover the relationships between cyclic di-GMP metabolizing proteins to further build understanding of the *S. coelicolor* cyclic di-GMP interactome.

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Abstract 2389

Profilin augments Cofilin induced Actin dynamics

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William Brieher

Spatiotemporal regulation of polymerization, depolymerization and regeneration of actin monomers is crucial for maintaining structure, motility, and stimulus response in eukaryotic cells. Both de novo production and treadmilling of actin are insufficient to maintain fast actin dynamics at physiologically relevant timescales, e.g., when a neutrophil is actively chasing a bacterium in a blood smear. The constant flux of actin monomers through diverse cellular networks is throttled by slow depolymerization rates which hinder timely generation of polymerization competent monomers. Cells employ plethora of actin binding proteins to modulate kinetics and dynamics of actin networks but most of them are not essential in yeast. We are searching for the minimum conserved mechanism powering fast actin dynamics in eukaryotic cells. We hypothesize that Profilin, a conserved actin monomer-binding protein, facilitates fast actin depolymerization in conjunction with known actin-severing protein, Cofilin. Using purified Profilin, Actin, and recombinant Cofilin we have reconstituted depolymerization reactions *in vitro*. By imaging pre-formed actin filaments coupled with measurements of severing and dissociation rates, we have characterized the role of Profilin in Cofilin mediated actin turnover dynamics. We show that Profilin enhances Cofilin mediated filament severing without displacing bound Cofilin from actin filament sides. Further, Profilin enhances Cofilin induced monomer dissociation at filament barbed ends. Using WT and K96AD98A mutant Cofilins we show that Profilin leverages two different actin-binding modes of Cofilin to destabilize actin filaments in distinct ways. Using bulk pyrene-actin polymerization assays we also determined that this cocktail of Profilin and Cofilin slows down initial polymerization rates but does not affect steady-state actin critical concentrations. Previous studies of bulk-phosphate levels have pointed towards Profilin and Cofilin working synergistically, but at opposite ends of the filament, to accelerate turnover. Our emphasis is on presenting macroscopic evidence of direct interplay between Cofilin and Profilin to enhance filament dissociation at the same end. Our work also differs from the established treadmilling model in showing that the barbed end of the filament is highly dynamic, especially in presence of Profilin and Cofilin at concentrations much lower that previously reported. In cells, Profilin and Cofilin are both recruited by the same phosphoinositide which can result in high local concentrations at specific sites on the plasma membrane, leading to rapid dismantling of actin barbed ends apposing these membranes. Insights from our work will pave the way for future studies on network specific turnover dynamics brought about by Profilin and Cofilin colocalization in physiological conditions.

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Abstract 2394

Structural Dynamics of a Hemolytic Protein

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Dan Grilley

A common technique utilized by pathogenic bacteria to infect hosts is the secretion of cytotoxic proteins, which break open host cells, exposing the bacteria to all the nutrients stored within. Proteus mirabilis, a gram-negative bacteria commonly associated with urinary tract infections, uses this very technique, secreting hemolysin A (HpmA) to infect hosts. Secretion of HpmA requires a second component, HpmB, located on the outer membrane of the cell. In this two-partner secretion pathway, HpmB couples the folding and transport of HpmA, allowing HpmA to achieve its folded and active form upon secretion. The proposed mechanism for this involves a Brownian Ratchet model, in which all energy for transport is derived from random motion of the protein within its environment. It is hypothesized that the β-helical HpmA is sequentially folded as it passes through HpmB, and that the folded segments serve as a mechanical stop, thus, ratcheting the protein down, preventing movement back into the cell. The β-helical structure of HpmA is characterized by circuits consisting of approximately 21 amino acids. To test if full circuits represent the mechanical stops, we have created a series of truncations variants that differ in length by a few amino acids. Using CD monitored denaturation experiments and functional experiments, we show that there are potential pause points of increased stability. To obtain a better understanding of the structural dynamics and stability of HpmA as they relate to the protein's mechanism of secretion, we are implementing NMR spectroscopy. The results of these various experiments will be discussed in the context of the Brownian Ratchet secretion model.

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Abstract 2396

Toward Determining the Effects of Hydrogen Orientation on Reduction Potential in Rieske Ferredoxins

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Benjamin LeVahn, Sangita Sinha, Christopher Colbert

Rieske Ferredoxins possess a unique type of [2Fe-2S] cluster that is coordinated by two Histidyl and two Cysteinyl residues. These proteins serve as important electron shuttles for various metabolic pathways. A key feature of Rieske ferredoxins is the wide range in reduction potentials when compared to standard [2Fe-2S] clusters; −150 to +320 mV compared to −450 to −250 mV respectively. Various factors affect reduction potentials in metalloproteins, chief among them being the metal/ligand interaction with the solvent and the position of the metal center with respect to ionizable or polar amino acids. It has been proven that the solvent exposure of the [2Fe-2S] cluster Histidyl ligands does not provide any significant influence on reduction potential, but what is unclear is how the electrostatic environment around the cluster is influenced by nearby amino acids. Biphenyl Ferredoxin (BphF) from Pandoraea pnomenusa B356 has been purified and crystallized via vapor diffusion, and an X-Ray crystal structure obtained at 1.53 Å. Key residues within the Cluster binding domain (CBD) have been identified when compared to BphF from Paraburkholderia xenovorans LB400 and mutated. The rate of electron transfer for wild type and mutated BphF is quantified by a Cytochrome C kinetic assay–Cytochrome C acts as a terminal electron acceptor with a measurable wavelength shift between its oxidized and reduced form. The high-resolution X-Ray crystal structure can accurately show the position/orientation of the peptide back bone, substituent groups, and [2Fe-2S] cluster geometry. It does not reveal hydrogen positions/orientations relative to the [2Fe-2S] cluster necessary to understand the partial charge distributions in the cluster environment, but neutron diffraction experiments would. However, growing protein crystals with sufficient volume (~0.1–1 mm^3) to be suitable for neutron diffraction is a major impediment. Therefore, we have employed, various crystallization techniques, ranging from vapor diffusion to batch crystallization, in order to produce a protein crystal sufficiently large enough for neutron diffraction. To date we have obtained crystals that are approximately 0.06 mm^3. Ultimately, this research aims to further the understanding of how the electrostatic environment around the Rieske [2Fe-2S] cluster affects the reduction potential and establish BphF as a model Rieske Ferredoxin.

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ASBMB

Abstract 2401

Applying the Brakes: Understanding the Role of Conformational Changes in Kinesin-5

Nelson Brown, *Xavier University of Louisiana*

Amaya Sanders, Kennedy Drake, Caleb Cook, Jordan Campbell, Kingston Robinson, Jenelle DeVry, Micquel Downs, Joseph Chaney

Human Kinesin-5 (Eg5) is an anticancer drug target and a molecular motor protein integral to the assembly of the bipolar spindle during mitosis. The neck-linker of Kinesin-5 is a 12–15 residue segment at the N-terminus that plays an important role in the processive transport of intracellular cargo along the microtubule surface. A deeper analysis of Kinesin-5 can unlock key details and the potential of these mechanisms. Although Kinesin-5 is an important anticancer target, much work is already completed on Kinesin-1. Kinesin-1 is one of the few kinesin motor proteins with a dimeric structure documented. This information allows us to have an idea of what Kinesin-5 may look like in form and provides a method for direct comparison. The goal is to determine the effects of insertions in the neck-linker of Kinesin-5 by investigating the catalytic activity, *in vitro*, and microtubule motion. The insertions are performed at different positions in the neck linker to determine whether more down-regulation occurs at different positions on the gene. These insertions are analyzed using ATPase assays to determine the activity of mutated proteins. Wild-type Kinesin-5 will be studied to compare the results with those of the mutated proteins. Our project seeks to give insight as to how the neck-linker controls structural asymmetry and initiation of the coil-coil domain in Kinesin-5. Our future goal is to test the response of Kinesin-5 to known Kinesin inhibitors and to generate a novel inhibitor.

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Abstract 2409

Utilizing ELISA to analyze the efficacy of Immunoglobulin E in MUC1 binding to support a novel approach to metastatic cancer drug development

Karly Guerrero, *California State University-Fresno*

Trevor Burlingame, Teresa Brooks, Cory Brooks

Immunoglobulin E (IgE) is an antibody produced by the human immune system in response to an allergy. These antibodies trigger the degranulation of mast cells releasing chemicals that cause inflammation and the symptoms associated with an allergic reaction. Recently, researchers have begun to employ IgE and the allergic response to specifically target cancer cells. The IgE in this study is 4H5-IgE, an antibody that binds the cancer associated protein MUC1. The efficacy of the 4H5-IgE antibody in response to treating metastatic cancers is being further studied by our collaborators at UCLA. The research we are currently conducting is to support this ongoing effort to develop 4H5-IgE as a novel treatment for cancer. We are currently working to troubleshoot an aspect related to quantification of the antibody being produced from Chinese hamster ovary (CHO) cells. An enzyme-linked immunosorbent (ELISA) assay has been developed to determine the amount of 4H5-IgE being produced from the cells. However, it was found that there was something getting in the way of the 4H5-IgE antibody binding to the recombinant antigen used in the assay. My project was to determine what was causing this phenomenon to occur. It was hypothesized that a matrix effect could be in play, which would cause inaccurate results from an ELISA procedure. It was observed that the measured concentrations of 4H5-IgE were unexpectedly high, and the results irreproducible under the original assay conditions. The first goal of our research was to determine an optimal concentration for the 4H5-IgE antibody, to produce a standardized curve that would serve as a reference moving forward. We ran a series of ELISA tests with 4H5-IgE as well as a positive control antibody, 3C6-IgE in PBS. We determined the concentration of antibodies that would produce the desired results. Next, we ran the same ELISA test with the optimized conditions, but instead of using the PBS buffer, we tested the hypothesis of the matrix effect by running the ELISA with the "matrix," ExpiCHO supernatant. Upon completion of a series of ELISAs, it was determined that the matrix effect could be a prime cause for the inaccurate results: something in the ExpiCHO supernatant is inhibiting the binding of the antibody, 4H5-IgE, to the target antigen. Further study within this research project includes assessing buffer exchange protocols and dilution approaches to minimize the matrix effect in the ELISA.

Thank you for the ongoing support from the Department of Chemistry and Biochemistry at California State University, Fresno. This project and presentation have been made possible by their Fall 2022 FSSRA funding. Special thanks to Dr. Brooks and his lab personnel, especially to Teresa Brooks for her guidance throughout the entirety of this project.

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Abstract 2411

Mathematical modeling and biochemical analysis support partially ordered CaM-MLCK binding

Melissa MacEwen, *University of Washington-Seattle Campus*

Domnita-Valeria Rusnac, Henok Ermias, Timothy Locke, Hayden Gizinski, Joseph Dexter, Yasemin Sancak

Activation of myosin light-chain kinase (MLCK) by calcium ions (Ca^{2+}) and calmodulin (CaM) plays an important role in numerous cellular functions including vascular smooth muscle contraction and cellular motility. Despite extensive biochemical analysis of this system, aspects of the mechanism of activation remain controversial, and competing theoretical models have been proposed for the binding of Ca^{2+} and CaM to MLCK. The models are analytically solvable for an equilibrium steady state and give rise to distinct predictions that hold regardless of the numerical values assigned to parameters. These predictions form the basis of a recently proposed, multi-part experimental strategy for model discrimination. Here we implement this strategy by measuring CaM-MLCK binding using an *in vitro* FRET system. This system uses the CaM-binding region of smooth muscle MLCK protein to link two fluorophores to form an MLCK FRET Reporter (FR). Biochemical and biophysical experiments have established that FR can be reliably used to analyze MLCK-CaM binding. We assessed the binding of either wild-type CaM, or mutant CaM with one or more defective EF-hand domains, to FR. Interpretation of binding data in light of the mathematical models suggests a partially ordered mechanism for binding of CaM to MLCK. Complementary data collected using orthogonal approaches that directly quantify CaM-MLCK binding further supports our conclusions.

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Abstract 2412

Exploring Effects of Mutations in the Kinesin-5 Neck-Linker

Caleb Cook, *Xavier University of Louisiana*

Joseph Chaney, Nelson Brown, Kennedy Drake, Amaya Sanders, Thandiwe Bush, Jordan Campbell, Carolyn Scruggs-Webster

Human Kinesin-5 (Eg5) is a motor protein that is an anticancer drug target as a result of its importance during mitosis. During mitosis, it is responsible for guiding the mitotic spindles towards their target location and studies that have inhibited the protein led to human cells undergoing mitotic arrest, unable to perform mitosis. An important region of study within Kinsein-5 is the 12–15 residue segment called the neck-linker region. If it is possible to safely inhibit Kinesin-5, it may become possible to create more focused and safe anticancer drugs because of cancer's reliance on mitosis. However, we do not fully understand the mechanisms behind the conformational changes of the Kinesin-5. As a result, we can make use of our much deeper understanding of Kinesin-1 as more information on it is documented in the Protein Data Bank compared to Kinesin-5. Kinesin-1 is our only and best model to compare with Kinesin-5 as both proteins share conserved residues, especially in the neck-linker region. By using this information, we can create point mutations in the neck-linker of Kinesin-5 and monitor the effect on the protein's activity. We make use of *E. coli* cultures in order to replicate many copies of the mutated protein. The mutations are designed to be at shared conserved residues between the neck-linker region of Kinesin-1 and Kinesin-5 in order to compare how the changes in structure effect the ability of the proteins to make conformational changes to perform their function. If it is possible to better understand the role of specific residues have in the function of Kinesin-5, it may be possible to create more efficient anticancer drugs.

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Abstract 2426

Molecular evolution of the α-domain in ascomycetes: and stable HMG-box DNA binding motif and an evolving C-Terminal extension

Doba Jackson, *Huntingdon College*

Abigail Cody

Objective: The objective of our study is to find the evolutionary relationship or the structural drift between related ascomycete yeast alpha domains.

Methods: The methods are bioinformatics, comparative molecular modeling, and abinitio molecular modeling using multiple programs.

Summary of Results: The α-domain consist of an HMG-box motif that participates in DNA binding and a C-terminal helical extension. The C-terminal helical extension does not have any significant homology to other eukaryotic proteins outside of the α-domain in yeast. So far, our studies have determined that the basic amino acids of the N-terminal loop, the intercalating hydrophobic residues of the first helix, and the hydrophobic residues required for interactions within the core of the protein are remarkably conserved in α-domains and HMG-box proteins. Our generated molecular models suggest that the first and third helix will be shorter and that the HMG-box core. Deletion mutants generated from the α-domain have demonstrated that the C-terminal helical extension (20–30 amino acids beyond the HMG-box motif) is required for structural stability *in vitro*. Structural models generated by comparative modeling and ab initio modeling reveal that this region will add two or more additional α-helices and will make significant contacts to helix III, II and I of the HMG-box core. The models we have developed are comparable to the predicted structure of the saccharomyces cerevesiae MATα1 determined by Alphafold.

Conclusions: We were able to illustrate how the extended α-domain would bind to DNA by merging of the α-domain and the LEF-1/DNA complex. Our α-domain/DNA binding complex places the N-terminal domain in the general region required for interaction of MATα1 binding partners such as MCM1. It is anticipated that our results will help understand protein domain evolution and how proteins can structurally evolve to interact with multiple binding partners and regulatory DNA sequences.

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Abstract 2428

Structural investigations of rubrerythrin from *B. pseudomallei*: Metals, domain swapping, and other interesting features of this ferritin-like superfamily protein

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Ruberythrin (Rbr) proteins belong to the ferritin-like superfamily and often function physiologically in oxidative stress tolerance, especially in anaerobic bacteria. Most Rbrs contain a di-iron site within a four-helix bundle with an N- or C-terminal rubredoxin-like domain. Rbr from *Burkholderia pseudomallei*, however, is missing the rubredoxin domain and also possesses unique structural features, including a domain swapped dimer. *B. pseudomallei* is an aerobic, Gram-negative soil-dwelling bacterium that is the causative agent of meliodosis. Given its association with high mortality rates, intrinsic antibiotic resistance, and ease of aerosol spread, *B. pseudomallei* has been identified as a potential bioterror agent. Ongoing questions that our work address include: What are the metals in the di-metal site in BpRbr? What is the physiological function of Rbr in aerobic bacteria like *B. pseudomallei*? What are the potential evolutionary implications of the differences between aerobic and anaerobic rubrerythrin proteins? What are the functional implications of the domain swapping in BpRbr? We will present our recent structural work on BpRbr in various metalated states, as well as in the presence of peroxide.

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Abstract 2430

The Interaction of a Nutrient Sensor with a Cytidine Deaminase at the Growth-to-Development Transition of *Dictyostelium discoideum*

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Alexandra Tyler, Aurora Coleman, David Lindsey

UbpA, a ubiquitin processing protease, is required for the growth-to-development transition (GDT) of the social amoeba Dictyostelium. UbpA, a homolog of yeast Ubp14 and human IsoT/USP5, functions to disassemble free polyubiquitin chains. Although ubpĀ cells grow normally, they are unable to make the GDT and they accumulate ubiquitin conjugates and misregulate genes at the GDT. The link between the biochemical function of UbpA and the UbpA-dependent pathways at the GDT is not yet clear. In Dictyostelium, development occurs as a response to starvation and UbpA is required for expression of LmcB, a nutrient sensor necessary for the GDT. We previously showed that LmcB binds to LmcA and CdaA, a putative cytidine deaminase. Here, we show that *E. coli* expressed His-tagged CdaA has cytidine deaminase (CDA) activity by using a colorimetric assay we adapted for Dictyostelium. LmcB is not present in ubpĀ cells at the GDT as in wild-type cells. Since LmcB is a putative nutrient sensor, and cells downregulate proliferation upon starvation, we investigated the response of ubpĀ cells to stationary phase chalones and found that proliferation of ubpĀ cells is reduced in the presence of stationary phase conditioned medium (SPCM); however, neither wild-type nor ubpĀ cells responded normally to SPCM from ubpĀ cells. This suggests that UbpA-dependent pathways are needed to generate chalone-like substances. To further identify and explore UbpA-dependent pathways and interactions, we used restriction enzyme mediated integration to mutant ubpĀ cells and then screened for second-site suppressors of the ubpA mutation. Three putative suppressor mutations were identified that resulted in partial rescue of the developmental defects of ubpĀ cells. These included mutations in bzpI, rasW, and an uncharacterized reading frame. In addition, site-directed mutagenesis of pufA, a negative regulator of development, also partially rescued the ubpĀ phenotype. These results will lead to a better understanding mechanisms cells use to sense starvation and carry out the GDT.

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Abstract 2433

Delineating the Events that Initiate Regulated Intramembrane Proteolysis by the Site-1 Protease, Prc, During Cell Surface Signaling in *Pseudomonas capeferrum*

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Cell surface signaling (CSS) allows Gram-negative bacteria to transcriptionally regulate gene expression in response to external stimuli. Binding of extracellular iron laden siderophores activate transcription for a subclass of Ton-B dependent iron import systems. These CSS pathways involve three key components: an outer membrane transducer for sensing the extracellular stimuli; an inner membrane sigma regulator, which undergoes regulated intramembrane proteolysis (RIP) to transduce the signal from the periplasm to the cytoplasm; and an extracytoplasmic function (ECF) sigma factor, which activates transcription of target genes. Previously, we showed that the central sigma regulator is stabilized in the periplasm by interaction with the outer membrane TonB-dependent transporter/transducer via interaction with its N-terminal signaling domain (NTSD). Thus, the question remains "Is this interaction sufficient to prevent activation of RIP and how does RIP proceed once activated?" We set out to answer these questions using our model system, the *Pseudomonas capeferrum* pseudobactin BN7/8 (Pup) import system. In this system the outer membrane transporter/transducer is PupB, the inner membrane sigma regulator is PupR, and the ECF sigma factor is PupI. The site-1 and site-2 proteases involved in RIP for Pseudomonas CSS are Prc (aka. Tsp) and RseP, respectively. We determined the binding affinity of Prc for PupR and performed proteolysis assays in the presence and absence of the PupB NTSD. These experiments showed that PupR preferentially binds to the PupB NTSD, and this interaction prevents degradation of PupR by Prc. Further, using mass spectrometry, we showed that Prc sequentially degrades PupR to remove the C-terminal 85 residues. We also determined the 2.0 Å X-ray crystal structure of Prc-His6 S485A in the resting state as well as the 2.2 Å activated state structure of Prc-His6 K510A in the presence of a peptide derived from PupR, indicating how site-1 proteolysis proceeds after activation. Finally, we used small-angle X-ray scattering to determine the low-resolution structure of the complex between Prc-His6 K510A and PupR residues 110–325. Together these results provide significant insights into how CSS is activated by RIP following extracellular stimulus. First, the transducer:sigma regulator complex must be disrupted to initiate RIP. Second, once disrupted the site-1 protease sequentially degrades the sigma regulator, removing the final 85 residues. Therefore, our data provide novel biochemical and structural insights into CSS activation for iron import systems in Gram-negative bacteria.

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Abstract 2455

Characterization of the Stability of Fibroblast Growth Factor 19 (FGF19)

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Fibroblast growth factor 19 (FGF19) is a member of the FGF family. FGFs are a group of proteins signaling different pathways such as cell proliferation and angiogenesis. The unusual decreased affinity of FGF19 for heparin/heparan Sepharose confers to the molecule the ability to travel from the intestine to the liver where it inhibits bile acid synthesis. Previous studies have also shown the protein is involved in glucose and lipid homeostasis, which makes it a potential candidate in treatments against diabetes. The clinical applications of FGF19 are however hindered by its structural instability. In this study, different biophysical methods were used to determine the thermal and chemical stability of FGF19. DSC results indicated a melting point of 45°C which was compared to CD and fluorescence results that were respectively 57°C and 55°C. The molecule is also prone to urea unfolding and enzymatic digestion as shown by the trypsin digestion experiments. Additionally, the cell proliferation activity of FGF19 was also assessed in 3T3 cells and compared to canonical FGF1. Similarly to the other members of the family, FGF19 also has significant mitogenic activities.

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Abstract 2458

Defining the structural basis of ASCC2's specificity for K63-linked polyubiquitin chains using 3D NMR analysis

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DNA damage requires precise repair mechanisms that function in a timely manner to maintain genomic integrity. The ALKBH3-ASCC complex is a DNA alkylation damage repair complex that binds to polyubiquitin chains which are recruited to the damage site. ASCC2, the subunit in the complex that directly associates with the polyubiquitin chain binds 2 ubiquitins simultaneously. This binding requires specificity that is important to the complex interaction in the pathway and repair response. Although the residues on ASCC2 that interact with polyubiquitin chains are known, the orientation by which the ASCC2 subunit and ubiquitin interact appropriately for repair is unclear. We use 3D NMR to show how specificity is achieved by measuring distances between residues on ASCC2 and diubiquitin. Distance restraints are important for binding interactions and will determine the orientation if the proteins when bound in the pathway. 13C and 15N labeled ASCC2 is currently under 3D experiments with diubiquitin. Collected data will be analyzed for specific ASCC2 and ubiquitin distances which will inform the ASCC2-diubiquitin model.

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ASBMB

Abstract 2505

Ancestor of All Apoptotic Caspases

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Caspases are crucial protein that aids in the initiation and execution of apoptosis. The beginning and completion of apoptotic cell death are carried out by apoptotic caspases. These processes are primarily conducted through two subfamilies in apoptotic caspases which are the initiator and executioner caspases. For carrying out such an important process these proteins are highly conserved in both subfamilies stemming from their ancestor that divided into different subfamilies to carry out apoptosis more efficiently. The purpose of this study is to investigate ancestral caspases to determine how the protein could have diverged from the common ancestor into two different families of caspases. Using ancestral sequence reconstruction, a reconstructed ancestor of all apoptotic caspases was generated and used to examine the caspase's evolutionary pathway. The ancestral sequence reconstruction was carried out by compiling caspases sequences from a curated database Caspabase from all chordates. We compiled 200 sequences to input into IQ-Tree to generate 3 ancestors that differed by selecting different species sequences for each of the ancestors' databases. Molecular Dynamic simulations were carried out on the ancestors along with modern caspases to analyze their molecular dynamic differences and similarities under water and urea conditions. From these studies, we were able to determine specific residues important in maintaining intermolecular forces intact in the ancestral caspases structure from either unfolding or folding. These residues would aid the common ancestor to evolve into the different families of caspases of initiator and executioners.

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Abstract 2512

MK-STYX Regulates Stress Granule Formation through its DUSP Domain

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Cells cope with stressors in a myriad of ways, one notable example being stress granule (SG) assembly. These biomolecular condensates are liquid-liquid phase separated aggregates of mRNA and RNA-binding proteins, functioning as a stopgap on translation until the stressor has ceased. Ras GTPase-activating protein-binding protein 1 (G3BP1) is a nucleator of stress granule formation, and therefore its modulation can enable rapid assembly or disassembly of SGs. The pseudophosphatase MK-STYX [MAPK (mitogen-activated protein kinase) phosphoserine/threonine/tyrosine-binding protein] interacts with G3BP1 and decreases SGs. Here, we show that this interaction is a result of the DUSP domain of MK-STYX. HEK293 cells showed a decrease in the number of cells possessing SGs when co-expressing mCherry-DUSP and GFP-G3BP1 Whereas, this effect was not observed for HEK293 cells expressing mCherry-CH2. Co-immunoprecipitation experiments were performed with HEK293 lysates expressing G3BP1-GFP and mCherry tagged MK-STYX, CH2 or DUSP domain fragments Western blotting revealed that G3BP1 coimmunoprecipitated with wild-type MK-STYX as well as the DUSP domain, but not with the CH2 domain. Furthermore, computational predictions of the interactions between G3BP1 and either MK-STYX, DUSP or CH2 indicate that the DUSP domain has the largest affinity for G3BP1. Phosphorylated tyrosine studies revealed that the presence of MK-STYX or its DUSP domain decreased the amount of G3BP1 phosphorylation, compared to the CH2 domain. Tyrosine phosphorylation at residue 40 of G3BP1 is essential to SG assembly. These results suggest a mechanism of SG regulation through MK-STYX, in that the DUSP domain binds to and lowers the phosphorylation status of G3BP1 to inhibit SG assembly.

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Abstract 2531

Acetylation regulates the oncogenic function of KPNA2 in non-small cell lung cancer

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Karyopherin alpha 2 (KPNA2, importin α1) interacts with karyopherin subunit beta 1 (KPNB1, importin β1) to deliver classical nuclear localization sequence-containing cargo proteins to the nucleus, followed by translocation back into cytoplasmic compartments. Aberrant nuclear accumulation of KPNA2 expression has been observed in numerous cancer tissues. The Ser/Thr protein kinase AMPK has been reported to regulate the phosphorylation and acetylation of KPNA2 in enterocytes. However, the impact of these post-translational modifications on modulating KPNA2 nucleocytoplasmic distribution and its oncogenic role remains unclear. Using immunofluorescence staining and trans-well migration assay, we demonstrated that, unlike the accumulation of wild-type KPNA2 in the nucleus that promoted lung cancer cell migration, expression of KPNA2 acetylation mimics Lys 22 (K22Q and K22Q/S105A) prevented their nuclear localization and reduced cell migration ability. The cytosolic KPNA2 K22Q interacted with and restricted the nuclear entry of E2F1, an oncogenic cargo protein of KPNA2 in lung cancer. Interestingly, immunofluorescence staining and subcellular fractionation analysis revealed that the AMPK activator Ex229 promoted the nuclear export of KPNA2 S105A but not K22R/S105A. However, the p300/CBP inhibitor CCS-1477 abolished this phenomenon induced by AMPK activation, suggesting that p300/CBP-mediated acetylation of KPNA2 promoted the nuclear export of KPNA2 in lung cancer cells. Our findings collectively suggest that AMPK-p300/CBP axis positively regulates the acetylation status of KPNA2, which may promote nuclear export and suppress the oncogenic activity of KPNA2 in lung cancer.

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Abstract 2532

The Role of MK-STYX in Neuro 2A Cells

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MAPK (mitogen-activated protein kinase) phosphoserine/threonine/tyrosine binding protein (MK-STYX) is a pseudo-phosphatase in the MAPK phosphatase family. Unlike a typical phosphatase, MK-STYX does not feature catalytic activity due to the lack of a critical cysteine residue in the active signature motif, HC(X5)R. Previous understandings of MK-STYX have alluded to this making it "useless," but novel findings have proven its role in several vital cell processes, including neuronal development. MK-STYX positively regulates the differentiation of neurite-like outgrowths. A recent study utilized an over-expression of the protein in comparison to control cells to highlight its impact on neurite growth and size. The outcomes suggest that MK-STYX induces neurite-like outgrowth formation and enhances the effects of nerve growth factor, resulting in more frequent and longer neurite-like extensions in pheochromocytoma cell line 12 (PC-12), a neuronal cell model. With this knowledge, it remained necessary to confirm findings in a primary neuronal cell line to verify the function of MK-STYX. To determine whether MK-STYX has a similar impact on neuronal cells, we transfected N2a cells with expression plasmids PEGFP (control), GFP-MK-STYX, and GFP-MK-STYX (active mutant) F1. Cells were cultured and assessed under fluorescent microscopy to determine changes in morphology. Preliminary experiments have supported the hypothesis that the protein upregulates neurite outgrowths in N2a cells. This data is critical as understanding the functionality of MK-STYX will ultimately help advance medical therapies for conditions such as Alzheimer's and cancer.

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ASBMB

Abstract 2547

mRNA-induced conformational changes control MYC protein complex assembly

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In cells, a subset of proteins – the hubs – have many binding partners. Hubs are enriched in transcription factors and enzymes. Typically, hub proteins have intrinsically disordered regions (IDRs) and different IDR conformations enable the interaction with structurally-diverse partners. It is currently unknown how the stoichiometry of protein complexes involving hub proteins is regulated. A potential strategy could be localized translation in different cytoplasmic compartments. We are interested in the functions of a cytoplasmic condensate, called TIS granule (TG) network that is generated through assembly of the RNA-binding protein TIS11B together with its bound mRNAs. We used fluorescent particle sorting to determine TG-enriched mRNAs and observed that transcription factors and proteins with IDRs are preferentially translated in TGs compared with the cytosol. One of the TG-translated transcription factors is MYC, which has ~80 different protein interactors. By including or omitting the MYC 3′UTR in expression constructs, we can control whether MYC is translated in TGs or the cytosol. We observed that upon translation of MYC in TGs, MYC forms protein complexes that cannot be established when MYC is translated in the cytosol. However, the constitutive MYC interactor MAX bound to MYC protein regardless of its location of translation. Using *in vitro* reconstitution experiments, we found that TG-dependent protein complexes can only be formed *in vitro* in the presence of RNA. Using NMR spectroscopy, we observed that RNA binding to the MYC IDR changes the IDR conformation and makes the protein interaction interfaces in the IDR accessible for binding. RNA binds a new, widespread IDR RNA-binding domain that consists of an α-helix in a serine-rich sequence context. In summary, for proteins with IDRs, we discovered a new regulatory mechanism for protein complex assembly that is based on RNA-induced stabilization of low-abundance conformational states. Proximity between IDRs and conformation-inducing RNAs is necessary for subsequent protein complex assembly and is accomplished through translation in TGs or through engineered 3′UTRs.

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Abstract 2567

Selectivity determinants of structurally-conserved loops near the target-binding site of SH2 and SH3 domains using chimeric proteins

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Recognition of modified proteins plays a critical role in the signaling pathways, with certain proteins recognizing specific motifs to introduce the cascades. Src Homology 2 (SH2) domains bind phosphorylated tyrosine (pTyr) residues, and over 100 of these domains have been described within the human genome. The SH2 domain is of particular interest to cancer biologists, as mutations within SH2-containing proteins can lead to uncontrolled cell growth through dysregulated gene expression. The binding pocket of the SH2 domain consists of two a-helices and a b-sheet, connected by dynamic loops. Conserved arginine and histidine residues within the binding pocket of the SH2 domain coordinate the negatively charged phosphate group bound to a tyrosine residue on another protein, allowing exquisite recognition of the target protein. The first structure obtained of the Src-SH2 binding to a target showed its recognition of the motif pY-E-E-I; however, different SH2 proteins display variable selectivity for certain amino acid sequences in their target proteins. The loops between the secondary structure motifs impart the specificity of the SH2 domain, and these selectivity-determining loops are a key focus of this work. Here, we expressed and purified a number of chimeric SH2 proteins, where we transferred the sequence of these loops from various SH2 domains into that of Src. Using fluorescence polarization, we determined binding affinities for our chimeric proteins with engineered and endogenous sequences that target Src, as well as the SH2 domains where the loops originated. We also used structure prediction tools to analyze these complexes. We find that indeed, these loops play a major role in the selectivity determinants of SH2 domains and their ability to recognize peptide sequences. We followed up this work with preliminary studies investigating similar questions in the SH3 domains of Src and Abl, which recognize poly-Pro sequences and are also often involved in tyrosine kinase signaling pathways. Taken together, these studies reveal fundamental mechanisms of protein-peptide specificity.

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Abstract 2568

Structural and binding studies of the beta4-beta5 loop in *Streptococcus pyogenes* Sortase A

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Bacterial sortases are transpeptidase enzymes that facilitate the attachment of various proteins containing a cell wall sorting signal motif to peptidoglycan on the cell wall. They first perform a cleavage at a specific cell wall sorting signal (CWSS) and then a ligation step to secure the protein on the surface of the bacterium. Sortase-mediated ligation (SML) is a technique that harnesses the specificity of this system for protein engineering purposes and has a growing potential dependent on research that explores or alters the enzyme's compatibility with alternate substrates. Our lab is interested in building up the field's sortase "toolbox" by interrogating the structurally conserved loops of class A sortases and the role they play in target selectivity. Initially, these studies focused on the conserved β7-β8 loop. Results of chimeric mutant activity backed the existence and importance of three non-covalent interactions that previous structural comparisons suggested the β7-β8 loop participated in, one of which was a hydrophobic interaction with the lesser-studied conserved loop, the β4-β5 loop. Attempting to answer similar questions posed for the β7-β8 loop regarding its role in the enzyme's activity and selectivity, the first step was to analyze activity and structure of loop-swapped chimeras of *Streptococcus pyogenes* Sortase A (spySrtA). After prepping, purifying, and experimenting on each of the chimeras, assays revealed that there was hardly any enzyme activity with the substrate sequence LPATX, where the residue X = G, A, or S. These results show that the wildtype β4-β5 loop is crucial for spySrtA activity. X-ray crystallography is a powerful tool which allows the visualization of protein-peptide interactions that may inform the meaning behind activity assay results. Crystals of the loop swap mutants proved to be a challenge; however, we solved the structure of a restorative point mutation, R139H, in the *Enterococcus faecalis* swap. Efforts to investigate the β4-β5 loop are continued as we investigate new spySrtA mutants which will test the role of the first few loop residues, as well as one mutant which investigates the role of loop length. In addition, work toward optimizing crystal growth of important mutants is ongoing. With each of these structures, assays, and projects, sortases are brought further into the light. This research is continuously revealing structural determinants of substrate selectivity in an enzyme that is central to protein engineering applications through SML and is also being noted as a potential target for therapeutic intervention.

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Abstract 2584

Investigating the effects of non-motif residues on the binding affinity of CFTR and HPV16 to human PDZ domains

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PDZ domains are a category of small peptide binding domains, identified by their well-conserved structure of a five-stranded anti-parallel beta-sheet and 1–2 alpha helices. They are prevalent in multicellular organisms and play an integral role in cell signaling and trafficking, protein-protein interaction, cellular scaffolding, and more. The human proteome contains 200+ PDZ domains, which can be targeted by various diseases and viruses which often disrupt cellular networks for their own benefit. This process may be related to higher pathogenicity in said diseases, which therefore makes PDZ domains a target for therapeutic research. This study focuses on human PDZ domains which are bound by the C-terminal sequences of HPV16 E6 oncoprotein and cystic fibrosis transmembrane conductance regulator (CFTR). These PDZ domains most notably recognize 2 key motif residues, which HPV16 and CFTR have in common (Lysine and Threonine, where CFTR: VQDTRL and HPV16: RRETQL). Despite this, HPV binds to significantly more PDZs, making it a more "promiscuous" binding domain. We have investigated the impact that the other non-motif residues have on overall binding affinity and whether they may cause this difference in promiscuity. To investigate this, we expressed and purified human PDZ domains which exhibit preferential binding to either CFTR or HPV16, and tested their binding affinity via fluorescence polarization assays through the use of substituted peptides. These peptides mimic the C-terminal sequences of CFTR and HPV16E6 with single point amino acid substitutions to that of the other sequence. We saw little to no variation between the binding of the variant peptides and the original sequences to the PDZ domains which we tested. Our results indicate that there is not a single residue which is controlling the increased binding of HPV16 vs CFTR, but rather a compounded affect of them together as a whole.

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ASBMB

Abstract 2586

Light-sensitive phosphorylation regulates feedback inhibition and filament assembly of human IMPDH1 retinal splice variants

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Photoreceptor cells in the retina require abundant guanine nucleotides to support signaling pathways critical to their function. To satisfy such high purine demand, the retina relies upon de novo purine synthesis to supplement nucleotide levels supplied by salvage pathways. As the rate-limiting enzyme in de novo guanosine triphosphate (GTP) synthesis, inosine monophosphate dehydrogenase (IMPDH) is highly regulated by feedback inhibition, allostery, polymerization, and higher-order assembly into micron-scale filaments in cells. In most tissues, the IMPDH1 isoform is expressed at low levels, while IMPDH2 is upregulated in proliferating cells. However, in the retina, two tissue-specific IMPDH1 splice variants IMPDH1(546), which has a 37-residue C-terminal extension, and IMPDH1(595), which has the same C-terminal extension plus a 49-residue N-terminal extension, are the dominant isoforms. These variants are less sensitive to feedback inhibition by GTP compared to canonical IMPDH1, enabling the increased GTP synthesis required to meet the demands of photoreceptor signaling. Recent phosphoproteomic analyses of bovine retinas identified that phosphorylation of IMPDH1 at S477 preferentially occurs in the dark, but an S477D phosphomimetic mutation appeared to have no effect on catalytic activity or GTP regulation of IMPDH1(546) *in vitro*. Based on its location, we hypothesized that phosphorylation at S477 would disrupt polymerization and filament assembly, which allows the enzyme to resist GTP inhibition and remain active. Here, we utilized negative stain electron microscopy (EM) and cryo-EM to investigate potential structural changes in IMPDH1 retinal variants induced by S477D, as well as enzyme kinetics assays and fluorescence microscopy to measure *in vitro* function and filament assembly in living cells. Negative stain EM showed that S477D had no effect on polymerization of IMPDH1(546) but completely prevented polymerization of IMPDH1(595). We then solved a 3.2 Å cryo-EM structure of an IMPDH1(546)-S477D polymer, which had architecture similar to a canonical IMPDH1 polymer. However, a 3.2 Å cryo-EM structure of an IMPDH1 (595)-S477D free octamer revealed an unexpected conformation at the polymer assembly interface. Kinetics assays demonstrated that S477D re-sensitizes the enzyme to GTP inhibition, which would lower GTP output in the dark, when less de novo GTP production is needed. We then transfected HeLa cells with constructs expressing FLAG-tagged versions of IMPDH1(546) and IMPDH1(595), which showed that IMPDH1 (595)-S477D exerts a dominant-negative effect preventing filament assembly of endogenous wildtype IMPDH in living cells, providing an explanation for how phosphorylation at S477 regulates cellular GTP production by tuning the filament assembly and GTP inhibition of IMPDH1 retinal variants.

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Abstract 2593

Plastid glycyl-tRNA synthetase structures with bound tRNAGly: Recognizing the anticodon in two steps

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Two types of glycyl-tRNA synthetase (GlyRS) are known, the α_2 and the $\alpha_2\beta_2$ GlyRSs. Both types of synthetase employ a class II catalytic domain to aminoacylate tRNAGly. In plastids and some bacteria, the α and β subunits are fused and are designated as (αβ)2 GlyRSs. While the tRNA recognition and aminoacylation mechanisms are well understood for α_2 GlyRSs, little is known about the mechanisms for $\alpha_2\beta_2$/(αβ)2 GlyRSs. Here we present the cryo-EM structures of the (αβ)2 GlyRS from *Oryza sativa* chloroplast in complex with cognate tRNAGly. The set of structures reveals that the U-shaped β half of the synthetase selects its cognate tRNA in two steps. In the first step, the synthetase engages the elbow and the anticodon base C35 of the tRNA. In the second step, the tRNA rotates ∼9° toward the catalytic center. The synthetase probes the tRNA for the presence of anticodon base C36 and discriminator base C73. This intricate mechanism enables the tRNA to access the active site of the synthetase from a direction opposite to that of most other synthetases.

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Abstract 2606

Discovery, characterization, and redesign of potent antimicrobial thanatin orthologs from *Chinavia ubica* and *Murgantia histrionica* targeting *E. coli* LptA

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Thanatin from the spined soldier bug (*Podisus maculiventris*) has been reported as a potent antimicrobial peptide with antibacterial and antifungal activity. Thanatin is a 21-residue peptide that forms a beta-hairpin structure held together by a disulfide bond that is necessary for activity. Its activity has been most characterized against *E. coli* and shown to interfere with multiple essential pathways, such as the lipopolysaccharide transport (LPT) pathway which is comprised of seven different Lpt proteins A-G. Thanatin binds to *E. coli* LptA and LptD of the LPT pathway, thus disrupting complex formation in multiple places and inhibiting cell wall synthesis and microbial growth. Here, we performed a genomic database search to discover novel thanatin orthologs and construct a consensus sequence, characterized their binding to *E. coli* LptA using biolayer interferometry, and assessed their antimicrobial activity against *E. coli* using minimum inhibition concentration assays. We found that thanatins from *Chinavia ubica* and *Murgantia histrionica* bound tighter (by 3.6- and 2.2-fold respectively) to *E. coli* LptA and exhibited more potent antibiotic activity (by 1.9- and 2.2-fold respectively) than thanatin from *Podisus maculiventris*. We crystallized and determined the structures of thanatin from *C. ubica* (1.90 Å resolution), *M. histrionica* (1.80 Å resolution), and *P. maculiventris* (2.43 Å resolution) to better understand their mechanism of action. Our structural analysis revealed that residues A10 and I21 in *C. ubica* and *M. histrionica* thanatin are key to improving the binding interface with *E. coli* LptA, while residue R19 in *M. histrionica* thanatin is key to improving membrane permeability, thus overall improving the potency of thanatin against *E. coli*. Furthermore, we designed a stapled variant of *M. histrionica* thanatin that removes the need for a disulfide bond. Our stapled variant retains binding, although it worsens inhibition ∼6-fold. Our studies notably highlight how nature has evolved the sequence of thanatin to better target *E. coli*, but also provide an atomic level model of its potency. Our discovery also presents a library of novel thanatin sequences to serve as future scaffolds for broad antimicrobial therapeutics, such as our novel redesign of the *M. histrionica* thanatin scaffold.

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Abstract 2607

The Human RAP1 and GFAPε proteins increase γ-secretase activity in a yeast model system

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Objective: Alzheimer's disease (AD) is an age-related disorder that results in progressive cognitive impairment and memory loss. Deposition of amyloid β (Aβ) peptides in senile plaques is a hallmark of AD. γ-secretase produces Aβ peptides, mostly as the soluble Aβ40 with fewer insoluble Aβ42 peptides. The rare, early-onset AD (EOAD) occurs in individuals under 60 years of age. Most of the EOAD cases are due to unknown genetic causes, but a subset is known to be due to mutations in the genes encoding the amyloid precursor protein that is processed into Aβ peptides or the presenilins (PS1 and PS2) that process APP. RAP1(TERF2IP) is a telomeric protein that is responsible for maintaining genome stability. As cells replicate/age, its level decreases. It is found in cytoplasm as well as in nucleus. It's role in cytoplasm is unknown. Our study was designed to identify the interacting proteins of RAP1 and investigate its possible role in age-related diseases.

Methods: Identifying the interacting protein of RAP1 was done utilizing a yeast 2-hybrid screen. Interactions of various proteins were verified using *in vitro* co-immunoprecipitation. We modified and improved a reconstituted γ-secretase system in *Saccharomyces cerevisiae* to measure the γ-secretase activity levels. Aβ peptide production was measured using ELISA. All interactions were verified by using immunoblotting along with immunofluorescence microscopy techniques.

Results: We have identified GFAPε (glial fibrillary acidic protein-ε) as a protein that interacts with the telomere protection factor RAP1. RAP1 can also interact with PS1 alone or with both PS1 and GFAPε together *in vitro*. GFAPε co-precipitated with RAP1 from human cell extracts. RAP1, GFAPε and PS1 all co-localized in human SH-SY5Y cells. Using a γ-secretase system reconstituted in yeast, we found that RAP1 increased γ-secretase activity, and this was further increased by the co-expression of GFAPε. However, expression of GFAPε alone was not able to significantly affect γ-secretase activity. Conclusion: Our data show that the nuclear protein RAP1 has an extratelomeric role in the cytoplasm through its interactions with GFAPε and PS1. RAP1 increased γ-secretase activity, and this was potentiated by GFAPε. Our studies are the first to connect RAP1 with an age-related disorder.

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Abstract 2610

Modifications in SARS-CoV-2 N Linker Region Regulate RNA Interactions and Phase Separation

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The N protein of the SARS-CoV-2 virion is critical for viral genome packaging via RNA binding and regulation of viral transcription at the replication-transcription complex (RTC). The N protein can be divided into five main domains, and the central region is the linker, which is predicted to be primarily disordered and has not been heavily studied. The linker is Serine-Arginine Rich, which is phosphorylated at multiple sites by host kinases during infection, thereby promoting the N protein's role in viral transcription. Phosphorylation is a critical process for the regulation of many cellular processes and can provide recognition sites for binding complexes. In a study that examined the recognition of the SARS-CoV-2 N protein by the human 14-3-3 protein, the linker was found to contain critical phosphosites for 14-3-3 binding. The goals of this project are to determine the structure, dynamics, and RNA interactions of the Serine-Arginine Rich linker region. To accomplish this, we performed Nuclear Magnetic Resonance spectroscopy (NMR) experiments to analyze the structure of the linker region of the N protein and its ability to bind viral RNA. NMR confirms predictions that the linker is not entirely unstructured and it is able to bind RNA. The linker region of the N protein with phosphoserine incorporated at S188 was also examined via an NMR titration experiment with 1-1000 RNA. Compared to wild type, the incorporation of phosphorylation decreases binding. Other biophysical techniques such as Analytical Ultracentrifugation (AUC) and Multi-Angle Light Scattering (MALS) are used to identify the association state of the linker and the size of the resulting protein-RNA complex. We are currently working to biophysically characterize the structure, dynamics, and viral RNA binding ability of a mutation found in the Delta and Omicron variants: the R203M linker, which have been shown to enhance viral infectivity.

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Abstract 2612

Structural and functional insights into the role of human BCDX2 complex in RAD51-dependent homologous recombination by cryoEM

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Homologous recombination (HR) plays a crucial role in the repair of DNA double-strand breaks and DNA replication forks. Rad51 recombinase assembles into long helical polymers that wrap around the ssDNA tail at DNA break sites and plays a central and essential role in HR reaction. Additionally, several RAD51 paralogs, including the tetrameric complex of RAD51B, RAD51C, RAD51D, and XRCC2 (BCDX2), have been identified as mediators to promote RAD51 presynaptic filament formation. Depletion of any individual BCDX2 subunit reduces DNA damage-induced RAD51 foci formation and weakens DNA repair. Despite each subunit of BCDX2 complex sharing 20–30% identity at the amino acid level with RAD51 including conservation of key residues in the nucleotide binding motifs, unlike Rad51, none of the BCDX2 subunits harbor the ability to self-assembly into filaments. How the individual subunits of BCDX2 are arranged and details of the mechanisms by which they promote RAD51 presynaptic filament assembly are unknown, in part due to lack of detailed structural information. To gain insights into this process, we reconstituted, purified, and determined the structure of BCDX2 to a nominal resolution of 2.3 Å using single-particle cryo-EM. The structure reveals detailed structural information regarding complex architecture and provides significant functional insights. RAD51D interacts on either side of its ATP binding domain with the ATP binding domains of RAD51C and XRCC2. The NTDs of RAD51B, RAD51C, and RAD51D decorate the periphery of the complex with each NTD being in close contact with both the ATPase domain and NTD of the neighboring subunit, while XRCC2, which lacks an NTD, associates with the ATP binding domain of RAD51D and the NTDs of RAD51C and RAD51D. An extensive network of interactions is formed between RAD51C, RAD51D, and AMP-PNP at the RAD51C/RAD51D interface. In addition to providing insights into the molecular basis of nucleotide specificity, our cryoEM structure and functional analyses explain how RAD51C mutations found in ovarian cancer patients inactivate the HR mediator of BCDX2. Altogether, our structural and biophysical analyses shed light on the role of BCDX2 in HR and provide the requisite foundation for understanding how pathogenic mutations in BCDX2 impact upon HR.

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Abstract 2631

Expression of Recombinant hRORγ and Purification for Functional and Structural Studies

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The circadian rhythm is an internal biological clock composed of a set of autonomous oscillators that help regulate various physiologically functions such as sleep and wakefulness. Circadian dissonance can result in sleep disorders, neurodegenerative disease, metabolic syndrome, and increased cancer risks. Positive and negative transcriptional regulators work together in an interlocked time-delayed feedback loop to drive the circadian rhythm. For instance, Retinoic Acid Receptor-Related Orphan Receptors (RORs) are nuclear receptors that enhance the functionality and stability of the circadian rhythm. The naturally occurring flavonoid Nobiletin (NOB) was reported to have a strong affinity for human ROR gamma (hRORγ) increasing circadian amplitude. The atomic interaction between NOB and hRORγ has not been clearly established. This has constrained the future application of NOB as a promising circadian-interfering drug candidate to ameliorate associated diseases. This project aims to solve the three-dimensional atomic structure of the hRORγ/Nobiletin complex to better understand and explain their interaction. The gene of hRORγ has been cloned into bacterial plasmid to be expressed using *E. coli* BL21 cells. Systematic optimization has been performed for the expression to produce large quantity of recombinant hRORγ. The recombinate protein has been purified using affinity chromatography and size exclusion chromatography. Circular dichroism has been applied to confirm the correct folding of the purified recombinant hRORγ. Future directions include co-crystallize the recombinant hRORγ with Nobiletin following the structural determination using X-ray crystallography. The resulting structural data will provide insight into the mechanism by which Nobiletin enhances the circadian rhythm and lay the groundwork for future drug development.

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ASBMB

Abstract 2634

Binding Characteristics of EWSR1 Protein Domains to Various Nucleic Acids

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The development of Ewing Sarcoma and Angiomatoid Fibrous Histiocytomas is associated with the Ewing Sarcoma breakpoint region 1/EWS RNA binding protein (EWSR1). EWSR1 is part of the TET protein family and has multifunctional properties that include a transcriptionally active domain, central RNA recognition domain, and C-terminal zinc finger domain. The specific roles of EWSR1 in the cellular process including gene expression and RNA processing is not well understood. Because of the importance of the various processes that EWSR1 plays a role in, it is important to understand its binding characteristics with nucleic acids and other proteins in gene regulation steps. Thus, our goal is to characterize the interactions of the various protein domains within EWSR1 to advance the knowledge of the protein's role in cellular interactions. We expressed and purified the individual EWSR1 domains and combinations of domains and screened the binding ability of these variants with RNA and DNA molecules using gel electrophoresis mobility shift assays (EMSAs). Our preliminary results demonstrate that the various EWSR1 domains bind with different affinity to both various shaped DNA and RNA structures (i.e., hairpin loop, G-quadruplex, etc.).

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Abstract 2638

High-yield purification of the cytosolic purinosome enzyme phosphoribosylformylglycinamidine synthase (PFAS) from human Expi293F cells

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Purine nucleotides act as essential building blocks for DNA and RNA, energy storage and signaling molecules, and cofactors in many enzymatic reactions. Their de novo synthesis is critical for cells that rapidly proliferate or are deficient in purine salvage enzymes, as in the neurological disorder Lesch-Nyhan syndrome. The de novo purine synthesis pathway consists of ten reactions catalyzed by six enzymes. All six enzymes dynamically reorganize in the cytosol to form biomolecular condensates called purinosomes. When cellular demand is high, purinosomes reversibly assemble to increase metabolic flux through the pathway. The purinosome behaves like a phase-separated body despite the absence of any significantly disordered regions in the pathway enzymes, as shown by live cell fluorescence imaging. Our long-term goal, *in vitro* reconstitution of the purinosome from purified components, will allow us to investigate the molecular mechanisms of purinosome assembly and function. Difficulty in isolating intact purinosomes from cells necessitated the first significant step toward our goal: the purification of all six individual enzymes. Four of the six enzymes (GART, PAICS, ADSL, ATIC) have crystal structures in some ligand conditions. However, the first and third enzymes in the pathway, PPAT and PFAS, have no human structures. This is likely due to their insolubility when expressed in *E. coli*. In order to produce recombinant protein in the human cell line Expi293F, which is frequently used to isolate significant quantities of secreted proteins, we chose PFAS as a prototype for streamlining the protocol for cytosolic protein purification. Transient transfection of a plasmid encoding an N-terminal Twin-Strep-tagged PFAS protein driven by a CMV promoter provided optimal expression of soluble protein. We refined a protocol consisting of initial affinity capture, removal of the Strep-tag, and subsequent purification by size-exclusion chromatography. The protocol yields about 1.1 mg of highly pure, soluble PFAS per liter of Expi293F cell culture. The purity of the sample was confirmed by negative stain electron microscopy (EM) and single-molecule mass photometry. Using this material, we determined a high-resolution cryo-EM structure of PFAS in its apoenzyme state (3.2 Å). Our optimized purification protocol will be applied to produce high yields of purinosome enzymes with native post-translational modifications, which may be critical for *in vitro* purinosome assembly.

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Abstract 2642

Nascent proteome of leukemia cells in the hypoxic bone marrow microenvironment – role of TIAR and FMRP

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Chronic myeloid leukemia (CML) is a type of cancer that develops in the bone marrow (BM). Progression involves presence of malignant cells also in peripheral blood (PB). Thus CML cells function in different microenvironments. Elimination of leukemia cells from the hypoxic bone marrow niche remains a challenge for nowadays therapies. Following our previous studies of stress response and T-cell intracellular antigen-1 (Tia) proteins in CML, we characterized how translation and nascent proteome of CML cells is different in PB vs BM. In particular we studied how RNA binding proteins TIAR and the fragile X mental retardation protein (FMRP) contribute in leukemia cells to pro-survival effect of the bone marrow microenvironment. To study the impact of the hypoxic bone marrow microenvironment, human CML cells (K562, Lama-84) were co-cultured with fibroblasts from the bone marrow stroma (HS-5) in a hypoxia workstation (1.5% O2 and 5% CO2) for at least 72 h. Simultaneously, cells were maintained in in standard growth conditions (atmospheric O2 and 5% CO2). In hypoxic conditions CML cells displayed prolonged population doubling time. We studied effect of TIAR or FMRP knock-down in different conditions. Quantitative comparison of proteins in complex with TIAR and FMRP by TMT-MS revealed their interaction with ribosomal proteins that was sensitive to microenvironment conditions. Therefore, we determined mono-/polyribosome profile, efficiency of protein synthesis and degradation as well as cap- versus IRES-dependent translation. The results demonstrated that interaction with stromal cells reduced formation of polyribosomes displaying synergy to hypoxia itself. We found that unlike FMRP, TIAR silencing affected heavy polyribosome assembly and preference for IRES/cap-dependent translation. Furthermore, we analyzed profile of newly synthesized proteins by mass spectrometry using quantitative non-canonical amino acid tagging, that combines bio-orthogonal labeling of synthesized peptides (BONCAT) with stable isotope labeling by amino acids (SILAC). Nascent proteome analysis revealed major differences in cell physiology and the activity of various signaling pathways related to specific conditions. We found that TIAR and FMRP contributed to synthesis of different sets of proteins relevant for cells functioning in the given microenvironment, that could create bases for the observed therapy resistance. Considering that targeting of translation by homoharringtonine is one of the strategies approved for CML treatment we verified significance of TIAR and FMRP in the context of this treatment. We observed pro-survival effect of stromal cells, particularly under hypoxia. Silencing of TIAR modified sensitivity of cells in a way depended on the hypoxia and interaction with stromal cells. Altogether, our results point to the selection and regulation of mRNA translation by RNA binding proteins as an important component of microenvironment-dependent resistance of leukemia cells to pharmacological treatment.

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ASBMB

Abstract 2643

Arabidopsis FBS INTERACTING PROTEIN 1 (FBIP1): a WD40 repeat-like protein ubiquitylation target associated with plant stress responses

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The ubiquitin 26S proteasome system (UPS) is a eukaryotic pathway that targets proteins for degradation to facilitate various biological processes, including environmental stress responses and the cell cycle in plants. The UPS accomplishes target selectivity using E3 ligases, enzymes that determine protein specificity and catalyze the attachment of ubiquitin onto targets for degradation. One major type of E3 ligase is the SKP1-CUL1-F-box (SCF) complex, which uses F-box proteins as interchangeable substrate adaptors to select protein targets to be ubiquitylated and then degraded by the 26S proteasome. The *Arabidopsis thaliana* F-box protein F-BOX STRESSED INDUCED 1 (FBS1) is the substrate adaptor for SCFFBS1, a complex that is hypothesized to target proteins for degradation under abiotic stresses. We identified an Arabidopsis WD40 repeat-like protein, FBS INTERACTING PROTEIN 1 (FBIP1) that is a nuclear localized interactor of FBS1. It is possible that SCFFBS1 regulates stress responses in plants by ubiquitylating FBIP1 to activate a stress response, thereby making plants more able to survive adverse conditions. To determine if SCFFBS1 destabilizes FBIP1 in a 26S proteasome-dependent manner, we measured the fluorescence of YFP-tagged FBIP1 expressed in Nicotiana benthamiana in the presence of increased FBS1 abundance, then treated samples with the 26S proteasome inhibitor MG132. We found that the presence of FBS1 significantly reduced the YFP fluorescent intensity of FBIP1, and that treating the sample with MG132 rescues fluorescence, showing FBS1 destabilizes FBIP1 in a 26S proteasome-dependent manner. To assess whether FBIP1 is involved in plant stress responses, root morphology of Arabidopsis fbip1 fbip2 double mutants under different abiotic stresses is being investigated. Although FBIP1 does not appear to be involved in regulating salt stress or nitrogen starvation responses in roots, its interactions suggest it may be implicated in regulating plant development via the cell cycle. This work investigates a possible mechanism by which the UPS and SCFFBS1 E3 ubiquitin complex, through FBIP1, regulates stress responses in plants. Understanding how plants regulate stress responses could potentially contribute to developing resources that optimize plant yields in changing environments.

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Abstract 2649

Validating Interaction of Peroxiredoxins with FK506-binding Proteins 51 and 52 in the Androgen Receptor Heterocomplex in Prostate Cancer

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The progression of prostate cancer (PCa) is associated with signaling through the androgen receptor (AR) and is affected by two co-chaperone proteins, FKBP51 and FKBP52, which are part of the AR regulatory complex. These proteins have distinct roles in regulating AR-dependent gene expression and promoting PCa progression, despite sharing 70% of their amino acid sequence. To determine changes in gene expression profiles influenced by FKBP52, genomic studies were conducted to identify the androgen-dependent signaling pathways differentially regulated in the presence or absence of FKBP52. These studies demonstrated dysregulation of oxidative phosphorylation pathways in FKBP52-deficient PCa cells *in vitro*. Further research on protein-protein interactions revealed that peroxiredoxins, a family of antioxidant enzymes, were among the top 100 interactors for FKBP51 and FKBP52. These enzymes protect cells from oxidative stress by controlling levels of reactive oxidative species produced during oxidative phosphorylation. Our goal is to confirm the interaction between FKBP51 or FKBP52 and specific peroxiredoxins in PCa, and to understand their role in AR-mediated PCa progression. To this end, peroxiredoxins tagged with FLAG (or DYKDDDDK sequence) were overexpressed in 22RV1 prostate cancer cells and co-immunoprecipitated (Co-IP) to verify their interaction with FKBP51 or FKBP52 in response to androgen treatment (Dihydrotestosterone). Purified recombinant protein pull-down assays were performed to validate these interactions further. Our results validated the *in vitro* interaction of PRDX2, PRDX3, and PRDX4 with FKBP51, and the interaction of PRDX1, PRDX2, PRDX3, and PRDX4 with FKBP52, all of which were verified by western blot detection. The validation of this protein-protein interaction network will provide insight into the AR heterocomplex by expanding the established regulatory functions of the FKBPs in AR signaling. Elucidating the role of the FKBP-peroxiredoxin signaling pathway in oxidative damage resistance may uncover a novel therapeutic target against PCa progression.

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Abstract 2650

Tumor-related RhoA GTPase mutants interact with effectors in the GDP-bound state

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RhoA belongs to the Ras-homology (Rho) small GTPase family and is a master regulator for many aspects of cell functions. Recurrent RhoA mutations have been identified in several human cancers, particularly in leukemia/lymphoma and gastric cancer. Intriguingly, both gain-of-function and loss-of-function mutations of RhoA are present, suggesting that RhoA GTPase may have a more sophisticated role in cancer and requires more rigorous examination. Here, we have focused on two of the gain-of-function RhoA mutations identified in Adult T cell Leukemia/lymphoma (ATLL) at residue A161 (A161P and A161V) and aim to reveal the underlying mechanistic basis for their function by biochemical and structural analyses. We found that in contrast to conventional gain-of-function RhoA mutants, such as RhoAG14V and RhoAQ63L which affect the GTP-hydrolysis activity, RhoAA161P and RhoAA161V are both fast cycling with drastically increased nucleotide dissociation and association rates, but only slightly reduced GTP-hydrolysis activity. Crystal structures of GDP-bound RhoAA161P and RhoAA161V have been solved. While RhoAA161P displays impaired solvent-mediated interactions to the bound nucleotide, RhoAA161V has a more exposed nucleotide binding pocket compared to RhoAWT and RhoAA161P. Most interestingly, RhoAA161P and RhoAA161V can interact with effectors in the GDP-bound state. Further 1H-15N HSQC NMR study provides evidence of active population in GDP-bound RhoAA161V. The dynamic properties of RhoA switch regions are affected differently by the two mutations based on molecule dynamics (MD) simulations. Thus, RhoAA161V and RhoAA161P are fast-cycling mutants with distinct mechanisms, and both likely endow their GDP-bound state towards an active conformation. These findings allow a better understanding of the oncogenic role of RhoA mutations in human cancer and shed light on how changes in RhoA dynamic properties caused by mutations may affect its function.

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Abstract 2660

Molecular basis for actin polymerization kinetics modulated by solution crowding

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Actin polymerization drives cell movement and provides cells with structural integrity. Intracellular environments contain high concentrations of solutes including organic compounds, macromolecules, and proteins. Macromolecular crowding has been shown to affect actin filament stability and bulk polymerization kinetics. However, the molecular mechanism behind how crowding influences individual actin filament assembly is not well understood. In this study, we investigate how crowding modulates filament assembly kinetics using total internal reflection fluorescence (TIRF) microscopy imaging and pyrene fluorescence assays. The elongation rates of individual actin filaments analyzed from TIRF imaging depended on the types of crowding agents (polyethylene glycol, bovine serum albumin, and sucrose) as well as their concentrations. Further, we utilized the all-atom molecular dynamics (MD) simulations to evaluate the effects of crowding on diffusion coefficients of actin monomers during assembly. Taken together, our data suggest that solution crowding can regulate actin assembly kinetics at the molecular level.

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Abstract 2669

Off-axis power-stroke and force sensing features of myosin-IC revealed by high-resolution cryo-EM

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Myosin motor proteins utilize energy from ATP hydrolysis to perform diverse biological activities including muscle contraction, intracellular transport, membrane trafficking and cell division. Distinct from other myosins, myosin-I binds to phospholipid anchors and some are highly force sensitive. These properties are linked to specialized roles in membrane remodeling and intracellular trafficking, including insulin-stimulated exocytosis of the glucose transporter (GLUT4). Previously, cryo-EM studies of myosin-I family member, myosin-IB, linked the force-sensing behavior to docking of a class specific N-terminal region (NTR) between the motor domain and lever arm. However, the role of the NTR and other class specific myosin-I features remain incompletely understood. Here we use cryo-EM to investigate the structural basis of force sensing and motility in myosin-IC, where these behaviors are distinctly different. We observed that unlike myosin-IB, the myosin-IC lever swing has an off-axis component, providing a mechanism for the asymmetric gliding of actin filaments *in vitro*. At the end of the lever swing (rigor), the NTR docks between the lever arm and motor domain as was observed in myosin-IB. However, while myosin-IB NTR docking features predominantly hydrophobic interactions, myosin-IC utilizes a network of cation-pi and salt bridge interactions. We propose that these differences in NTR interactions differentially tune force sensitivity. This conclusion is supported by a high-resolution structure of actomyosin-IC ΔNTR (truncated myo-NTR) which reveals a much more mobile lever arm, consistent with a loss of force sensitivity observed in the mutant. Overall, these structures provide new molecular insights into the role of myosin-IC as a slow power generating motor.

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Abstract 2671

The ASCC2 CUE domain selectively recognizes K63-linked polyubiquitin chains by binding between adjacent ubiquitins and making unique interactions with the distal and proximal ubiquitin

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The ALKBH3-ASCC DNA repair complex is recruited to alkylation damage sites by binding to K63-linked polyubiquitin chains that are assembled in response to damage. ASCC2, a subunit of the ALKBH3-ASCC complex, utilizes its CUE domain to selectively bind K63-linked polyubiquitin chains with greater affinity than monoubiquitin or other types of polyubiquitin chain linkages. CUE domains generally do not exhibit a strong preference for specific types of polyubiquitin chains and the structural basis for the unique ubiquitin-binding properties of the ASCC2 CUE domain have not been clearly elucidated. Here we present a model of the ASCC2:K63Ub2 interaction based on SAXS, NMR, and mutagenesis data which suggests the ASCC2 CUE domain achieves specificity for K63-linked polyubiquitin chains by binding to two adjacent ubiquitin molecules simultaneously. The ASCC2 CUE domain binds between adjacent ubiquitin molecules within K63-linked polyubiquitin chains and makes unique interactions with both the distal and proximal ubiquitins. The preference of the ASCC2 CUE domain for K63-linked polyubiquitin chains provides another example of how the CUE domain can be adapted to modify its ubiquitin-binding properties for diverse biological functions.

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Abstract 2681

The structural basis for DNA-uptake by Acinetobacter

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Natural transformation is one of the fundamental mechanisms for horizontal gene transfer (HGT) in bacteria; it occurs through uptake of extracellular DNA (eDNA) by bacterial cells which can then incorporate the novel genetic material into their genomes. Bacteria from the genus Acinetobacter, which include environmental strains, commensals, and opportunistic pathogens, are universally naturally-competent, which is thought to contribute to the recent rise of multidrug-resistant (MDR) Acinetobacter infections. MDR strains now account for more than half of Acinetobacter infections. Natural competence in Acinetobacter is dependent upon DNA-uptake mediated by type IV pili (T4P). T4P are extracellular helical polymers composed of thousands of protein subunits called pilins. The majority of the fibers is composed of a single subunit (the major pilin). The mechanism by which Acinetobacter T4P bind DNA, including which subunits serve as eDNA-receptors, is unknown. To identify the structural mechanisms by which T4P in Acinetobacter take up DNA, we have recombinantly expressed Acinetobacter T4P subunits and directly measured their affinity for double-stranded DNA. We have purified Acinetobacter T4P subunits both individually and refolded a trimeric complex (PilX, PilV and PilW) though to reside at the pilus tip. We assessed their ability to bind plasmid DNA using EMSA (electrophoretic mobility shift assays). Preliminary data indicates that two soluble minor pilins, PilE1 and PilE2 show weak DNA binding *in vitro*, but show no obvious homology to known DNA-binding proteins. Additionally, we have measured T4P function in *Acinetobacter baumannii* transposon mutants of each of the eight putative T4P subunits and found that one, fimT, is not required for pilus assembly. A homologue of FimT has recently been proposed as a DNA receptor in Legionella pneumonia. Using structural models of T4P generated by AlphaFold, we are currently using computational approaches to predict the interface of DNA recognition in Acinetobacter T4P.

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Abstract 2688

Characterization of Site-Specifically Incorporated 3-Chlorotyrosine in Heterologously Expressed Ie Ribonucleotide Reductase

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In an effort to discover new routes of enzyme inhibition for treatment of bacterial infections, we aim to elucidate the mechanism of cofactor assembly and deployment in ribonucleotide reductases (RNRs) expressed in bacterial pathogens. RNRs are a large family of enzymes responsible for the biosynthesis of the building blocks of DNA in nearly all living organisms. They convert ribonucleotides to 2′-deoxyribonucleotides for DNA synthesis and repair. Most class I RNRs assemble a dinuclear transition metal cofactor in their beta subunit, but the most recently discovered subclass, Ie, does not require a metal ion for oxidation of its active site Cys residue to initiate catalysis. The Ie Cys oxidant is a 3,4-dihydroxyphenylalanine radical (DOPA·) generated from a 3-electron oxidation of Tyr123. Not directly relying on a transition metal for initiation of the ribonucleotide reduction mechanism is thought be an advantage for pathogens harboring Ie RNRs, allowing these organisms to counter transition metal sequestration by the host. The goal of my project is to understand how the DOPA· is synthesized from Tyr123 in Class Ie RNRs through incorporation of non-canonical Tyr analogs at this position. I have successfully replaced Tyr123 with 3-chloro-tyrosine in the Aerococcus urinae RNR Ie beta subunit using amber codon suppression. This substitution was expected to impede the installation of the hydroxyl group at position 3 in the tyrosine ring and subsequently affect the progression of cofactor assembly. Excitingly, my initial results show successful incorporation of the analog. After activation, a hydroxyl group is installed on the ring at position 5 despite the presence of the chlorine substituent at position 3, as confirmed by a 1.8 Å resolution X-ray crystal structure and intact-protein mass-spectrometry analysis. The 3-Cl-Tyr substitution also permits formation of a radical, albeit with a diminished EPR signal when compared to the wild-type Au RNR Ie beta subunit. The results obtained and described herein suggest that post-translational oxidation of Tyr123 in the Au RNR Ie beta subunit can proceed even with a chlorine substitution at the 3 position, but the stability and/or yield of the active cofactor is affected. This work sets the stage for obtaining a complete understanding of how Tyr substitutions impact class Ie RNR cofactor assembly. We anticipate that our efforts to uncover new mechanistic information about this important enzyme subclass will aid in the development of therapeutics targeting class Ie RNRs in bacterial pathogens.

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ASBMB

Abstract 2702

Highly synergistic combinations of nanobodies that target SARS-CoV-2 reduce viral load in hACE2 transgenic mice

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The ongoing emergence of SARS-CoV-2 variants threatens current vaccines and renders current therapeutic antibodies obsolete, demanding powerful new treatments that can resist viral escape. We therefore generated a large nanobody repertoire to saturate the distinct and highly conserved available epitope space of SARS-CoV-2 spike, including the S1 receptor binding domain, N-terminal domain, and the S2 subunit, to identify new nanobody binding sites that may reflect novel mechanisms of viral neutralization. Structural mapping and functional assays show that these highly stable monovalent nanobodies potently inhibit SARS-CoV-2 infection, display numerous neutralization mechanisms, are effective against past and present emerging variants of concern, and are resistant to mutational escape. Rational combinations of these nanobodies that bind dissimilar sites within and between spike subunits exhibit extraordinary synergy and suggest multiple tailored therapeutic and prophylactic strategies. All mouse involved experiments were performed in compliance with the Institutional Animal Care and Use Committee and mice were housed and maintained in a specific pathogen-free conditions at Seattle Children's Research Institute. Infected mice with SARS-CoV-2 were housed in a Biosafety Level 3 facility in an Animal Biohazard Containment Suite. Prophylactic intranasal application of a synergistic pair of unmodified nanobodies in 10–12 week-old female K18-hACE2 transgenic mice, a mouse model of SARS-CoV-2 infection, showed significant reduction in viral load after 3 days post-challenge with SARS-CoV-2, the first demonstration of synergy *in vivo*. In summary, our results show that our diverse repertoire of nanobodies can neutralize current variants of live SARS-CoV-2, pairs of nanobodies that bind distinct sites on spike show tremendous synergy in neutralizing efficacy *in vitro*, and the application of synergizing pair of nanobodies translates to an *in vivo* mouse model of SARS-CoV-2.

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Abstract 2704

Elucidating protein-protein interactions of Drosophila UNC-45 via *in vivo* and *in vitro* chemical crosslinking

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UNC-45 is a protein that has been observed to play many roles in the development and differentiation of various kinds of tissues. Across different species, missense mutations and null mutations of UNC-45 have been the cause of disorganized muscle assembly and intestinal cell development, retarded eye development, and increased cancer proliferation. With a structure that does not hint at any obvious catalytic activity, the most likely mechanism for UNC-45 functions is as an essential scaffold mediating protein-protein interactions. However, the number and type of confirmed protein-protein interactions for UNC-45 does not reflect its involvement in such diverse roles during the development and differentiation of various tissue types – with the short list consisting of microtubules, HSP90, HSP70, myosin, and itself. By using formaldehyde to perform *in vivo* chemical crosslinking of *Drosophila melanogaster* pupae in an effort to capture the transient and low affinity UNC-45 protein-protein interactions we were able to identify conditions that produced chemically crosslinked protein-protein complexes of UNC-45 with minimal fixation of the whole pupae. Our experiments indicate that *in vivo* protein-protein interactions can be captured in a whole living organism as small as a Drosophila pupa. Chemically crosslinking protein-protein interactions in their native environment is a more direct approach to identifying physiologically relevant protein-protein interactions than yeast two-hybrid assays or *in vitro* biophysical assays. After optimizing the procedures and conditions for generating UNC-45 binding protein conjugates, we intend to proceed with immunoprecipitation and high-resolution mass spectrometry to identify the proteins that complexed with UNC-45 *in vivo*. In a parallel attempt to elucidate the interaction sites between a previously reported protein-protein interactions involving UNC-45, *in vitro* chemical crosslinking of purified mixtures of UNC-45 have been performed. Using formaldehyde for *in vitro* chemical crosslinking, Drosophila UNC-45 has been determined to form 2-mer to 4-mer oligomers at concentrations as low as 12μM. This concentration is significantly lower than the 100μM concentration previously reported in the literature when UNC-45 had a single residue replaced with a photocrosslinker, thus indicating there are other unidentified interactive faces along UNC-45 that contribute to the formation of oligomers. As a result, the previously proposed mechanism of UNC-45 oligomers building the sarcomere could be wrong. We are in the process identifying these UNC-45 interactive faces using transmission electron microscopy. We are also in the process of elucidating the interactive mechanisms between microtubules

and UNC-45, and between UNC-45 and HSP83, a HSP90 Drosophila homolog.

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Abstract 2705

Structural ribosome profiling of exponential stage *Pseudomonas aeruginosa* cells reveal an ensemble of ribosome assembly states and conformations

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Pseudomonas aeruginosa is an opportunistic pathogen that causes chronic biofilm infections. Many antibiotics target protein biosynthetic activity but are ineffective at clearing *P. aeruginosa* biofilm infections. Biofilms contain bacteria that are in a variety of different physiological states, including subpopulations of active cells and dormant cells that are antibiotic-tolerant. Here, we report our initial structural studies on a crude ribosomal fraction quickly isolated from log phase cells with a mixed bed ion exchange resin. In anticipation of particle heterogeneity, a large data set was collected by cryo-EM that resulted in nearly three million good particles. Using a data processing pipeline that utilizes 3-dimensional variability to sort particles in silico, we identified at least four unique assembly states, some with distinct conformations. We quantified the relative number of particles present in each state. Specifically, we found three different assembly states for the 70S ribosome, including ribosomes with a single tRNA in the E site (E), tRNA in both the P and E sites (PE), and in all three sites (APE). In addition, the E-site assembly state was present in three distinct conformations. We also identified two conformationally distinct structures of the 30S subunit, and two conformationally distinct structures of the 50S subunit. Four of these structures are views of the *P. aeruginosa* ribosome that have not been previously reported, demonstrating the value of working with crude ribosome preparations to determine their structural profiles. Current work focuses on comparing the distribution of ribosome states described here to those found in dormant *P. aeruginosa*, to better understand the role of ribosome hibernation in the tolerance of *P. aeruginosa* biofilms to ribosome-targeting antibiotics.

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Abstract 2710

Probing of Caspase-6 Structure, Inhibition, and Dynamics for the Development of Neurodegeneration Therapeutics

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Jasna Fejzo, Jeanne Hardy

Cysteine aspartate proteases, caspases, are a family of apoptotic and inflammatory human proteases implicated in a variety of diseases. Specifically, increased expression and activity of caspase-6 in human neurons has been associated with common neurodegenerative disorders including Alzheimer's Disease (AD) and Huntington's Disease (HD). In AD, caspase-6 cleavage of microtubule-associated proteins tau and tubulin can lead to axonal degeneration and subsequently the observed cognitive decline. The role of caspase-6 in these degenerative pathways, as well as its minimal importance in homeostatic functioning, suggest it may be an optimal drug target. In our research, we delve into structural aspects of caspase-6 that may render it susceptible to selective inhibition over other caspases. Previous structural biology investigation as well as high-throughput screening coupled with structure-activity relationship optimization have uncovered unique structural interconversions and nanomolar caspase-6 inhibitors. Here, I expand upon our understanding of caspase-6 structure and regulation utilizing Nuclear Magnetic Resonance (NMR) to gain mechanistic insight. Using isotopic methyl-labeling strategies we can further elucidate the 130's helix-strand interconversion associated with substrate's binding to the proteolytic active-site. Understanding the manipulation of the enzyme into its active or inactive conformations may provide one route of selective inhibition. Complementarily, the sensitive detection afforded by NMR can determine the structural orientation of our previously identified inhibitor within its allosteric pocket. Collectively these endeavors contribute to our growing understanding of the biochemical role of caspase-6 in AD pathophysiology and progress toward development of treatments for neurodegeneration.

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Abstract 2712

The Cytotoxic Mechanism of Killer Protein 4-Like proteins in *Saccharomyces cerevisiae*

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Crop loss and spoilage caused by fungi cost the global economy over $60 billion annually. The fungus *Ustilago* maydis produces an antifungal protein known as Killer Protein 4 (KP4) that has been used against crop pathogens, including corn smut, head blight in wheat, and black rot. Homologs of KP4 (KP4-like; KP4L) have been identified in many species of fungi and could be a potential resource for new antifungals, but their activities remain largely uncharacterized. Molecular dynamics simulations suggest that KP4L proteins adopt the same structure as KP4, suggesting similarities in their modes of action, i.e., calcium channel interference. Eight KP4L proteins were used to transform the model yeast *Saccharomyces cerevisiae* to study their expression and function. Surprisingly, the induction of a KP4L gene isolated from the filamentous fungus *Basidiobolus meristosporus* (KP4-Bm) caused a reversible growth arrest at the G1/S phase transition in *S. cerevisiae* that was dependent on the presence of a signal sequence for endoplasmic reticulum translocation. Moreover, a whole genome screen for suppressors of KP4-Bm growth arrest identified four genes involved in endosomal trafficking, suggesting that the toxin was exported to the cell surface. Epitope tagging of KP4-Bm enabled its detection in the extracellular milieu by Western blotting, further confirming extracellular export. Deletion of the KP4-Bm N-terminal signal sequence also blocked extracellular export. Finally, excess calcium and other cations in growth media were found to suppress KP4-Bm growth arrest, suggesting a similar mechanism of action. These results indicate that KP4L proteins are extracellular toxins with a similar mode of action to KP4. Therefore, KP4L proteins could represent an untapped resource for the future development of strategies to mitigate fungal disease, such as fungal-resistant transgenic plants.

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Abstract 2713

Hydrophobic interactions and hydrogen bonds are key to the chaperone activity of HdeB with client proteins

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HdeB is an acid-stress chaperone that plays a crucial role in the survival of pathogenic bacterial by protecting other bacterial ("client") proteins from aggregation and facilitates subsequent refolding upon pH neutralization. HdeB is active and highly dynamic near pH 4. Since HdeB doesn't undergo significant structural changes at pH 4, it is thought that its function derives from its intrinsic flexibility. A deeper understanding of how HdeB functions is required to disrupt the chaperone's activity and mitigate the harm caused by those pathogenic bacteria, which cause dysentery. To investigate the function of HdeB, the client protein ModA was specifically chosen due to its small size (22.5 kDa), making it suitable for NMR spectroscopy, our primary experimental method. We probed the interaction between HdeB and ModA with 1H-15N correlation NMR experiments (1H-15N HSQCs). For our first set of experiments our spectra monitored HdeB, which was labeled with the NMR-active isotope 15N; each peak in this spectrum represents a backbone amide for each residue in HdeB. By monitoring chemical shift changes in the HSQC spectra of 15N-labeled HdeB by itself (apo) compared to a mixture of HdeB and ModA (holo) at pH 4.5, we were able to determine which HdeB residues participate in client binding. This comparison confirmed HdeB may interact with ModA in several regions: the N-terminus, the space between the dynamic BC loop (residues 31–43) and the C-terminus, the region between helices A and C of HdeB. To determine whether these sites of interaction are universal or specific to each client protein, we recorded an HSQC spectrum of 15N-labeled HdeB with another client protein, HisJ. Comparison between the spectra of HdeB in the presence of the two client proteins showed that the interaction sites are largely the same, suggesting that HdeB may interact with most client proteins in the same way. We also completed the chemical shift assignment of 15N,13C-labeled ModA, which allowed us to characterize which residues in the client interact with HdeB. ModA residues with significant chemical shift changes from apo to holo (with HdeB present) consist largely of amino acids that are hydrophobic or have side chains that can form hydrogen bonds; these are the same type of amino acids that exhibit significant chemical shift changes in HdeB. This similarity indicates binding between HdeB and its client proteins is dependent on hydrophobic interactions and hydrogen bonding.

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Topic Category RNA: Processing, Transport, and Regulatory Mechanisms

Abstract 1211

Identification and functional analysis of Piwi-interacting RNAs in cervical cancer

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piRNAs are small non-coding RNAs that interact with the Argonaute protein Piwi to form the Piwi-piRNA complex. Several studies have shown the aberrant expression of piRNAs in the germline as well as somatic tissues. In addition, several reports indicate a significant role of these piRNAs in various cancer types. Our study focuses on identifying piRNAs that are exclusively and/or differentially expressed in cervical cancer. An in silico approach was used to predict piRNAs from small RNA sequencing data of cervical cancer tissues obtained from NCBI SRA (SRP119662). FastQC analysis was carried out for all 56 samples (32 control and 24 diagnosed), which revealed good quality base calls. The 3′adapter sequences were trimmed, and the remaining reads of 5 to 45 nucleotides were mapped against the human genome (hg19). After filtering unaligned reads and known small RNAs, reads with 26–31 nucleotides length were matched against the piRNA bank and piRBase sequences for the prediction of piRNAs. Based on its read expression, a few significant piRNAs were selected, and their expression was further validated in cervical cancer cell line. Interestingly, piRNAs, hsa_piR_019324 showed very high expression in CaSki in comparison to the control. Further, to investigate whether the overexpression of hsa_piR_019324 was associated with cervical cancer progression, we inhibited the piRNA expression using anti-sense oligos in CaSki cells and investigated its potential role in malignancy by employing several methods such as MTT assay, colony formation assay, sphere formation assay, transwell invasion and migration assay. We observed that the knockdown of hsa_piR_019324 significantly inhibited CaSki cell proliferation, migration, invasion, and colony-forming ability. In summary, this study revealed that hsa_piR_019324 is involved in cervical carcinogenesis and hence could be a promising prognostic marker and therapeutic target for cervical cancer.

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ASBMB

Abstract 1221

Interplay between TREX-2 subunits an the TPR nucleoporin in mRNA export

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The TRanscription and EXport 2 (TREX-2) complex is a key player in mRNA maturation. TREX-2 acts as a bridge between the transcription and export machineries and the nuclear export machinery, including nuclear pore complexes (NPCs), the channels through which mRNA leaves the nucleus. The TREX-2 complex is well conserved among eukaryotes; in humans, it consists of GANP, PCID2, ENY2, DSS1 and CETN2/3 proteins. We have previously applied an Auxin-Induced Degron (AID) strategy to analyze the interactions of TREX-2 at the NPC, and discovered that the depletion of the nucleoporin TPR leads to altered transcriptomic patterns that closely mimic the patterns after GANP depletion, as well as loss of interactions between TREX-2 and the NPC. Here, we have further analyzed the functions of individual TREX-2 subunits (CETN2, ENY2, GANP, PCID2) using AID-tagging and selective degradation. Our findings suggest that loss of TPR, GANP and PCID2 cause closely related patterns of disruption for gene expression, while the changes that result from CETN2 and ENY2 are distinct from those subunits and from each other. We are currently working to understand the molecular mechanisms that underlie the functional distinctions among the TREX-2 subunits.

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Abstract 1237

Interaction Between the Viral RNA Leader Sequence and nsp1 in SARS Coronavirus

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Nonstructural protein 1 (nsp1) of severe acute respiratory syndrome coronavirus (SARS-CoV), inhibits host translation thorough cleaving host mRNA and blocking the translation initiation site on the 40S ribosome. Stem-Loop-1 (SL-1) of the viral RNA leader sequence has been identified to bind to nsp1, allowing viral RNA to escape translation repression. However, the specific residues on nsp1 and the specific sequences on SL-1 important to binding have not been experimentally verified. To investigate this binding, we used gel-shift assay and RNA pull-down to verify binding between nsp1 and SL-1. By mutating SL-1, we seek to identify the nucleotides of SL-1 that bind to nsp1. Based on recent literature, we hypothesized that disrupting the stem region of SL-1 will decrease binding between nsp1 and SL-1. Moreover, we seek to identify the residues important to binding to SL-1 by mutating specific amino acids of nsp1. Interestingly, nsp1 is a small protein (180 amino acids) with intrinsically unstructured regions at both C- and N-terminal ends of the protein. Based on recent literature we hypothesize that disrupting the R124 and K125 residues will decrease binding to SL-1. The results of this study will increase the knowledge of how viral RNA is able to escape suppression of host gene expression. To investigate the binding of nsp1 to SL1, we used nsp1 purified from bacterial lysate using glutathione beads followed by precision protease cleavage of GST-nsp1, and biotinylated RNA. LightShift Chemiluminescence RNA EMSA Kit (Promega) was used to detect the RNA in complex with nsp1 using a gel shift assay. Contrary to our hypothesis, we found an increase in nsp1 binding to the RNA carrying stem mutation, and a decrease in nsp1 binding to the RNA with the loop mutation. Moreover, we observed two distinct bands in the stem mutant indicating two possible binding sites on SL-1. Using an electrophoretic mobility shift assay, the loop region of SL-1 has been determined to be vital for binding to nsp1 *in vitro*. We hypothesize when the stem was mutated, we created a new binding site for nsp1. Currently we are further investigating several mutations in SL-1 to identify the actual binding site.

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Abstract 1238

The Role of SARS-CoV Non-structural protein 1 in Regulating RNA stability

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infected more than 600 million people across 219 countries during the past three years. SARS-CoV-2 consists of a positive-strand RNA genome that encodes structural and nonstructural proteins and shares a 79% sequence homology with severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1). Nonstructural proteins are necessary for viral replication and suppression of the host cell immune response. Nonstructural protein 1 (nsp1), a small protein conserved among most beta-coronaviruses, inhibits host messenger RNA (mRNA) translation by binding to ribosomal mRNA channels. Nsp1 also triggers degradation of host mRNA while viral RNA remains intact. We have previously shown that nsp1 localizes within stress granules (SGs), non-membranous vesicles of stalled mRNA that form in response to viral infection. We also found that upon induction of stress, SGs disperses within 60–120 minutes in the presence of nsp1. Since SGs are known to store and protect translationally stalled mRNAs that are target of nsp1, we sought to analyze the level of mRNAs accumulation in SGs in the presence of nsp1. The goal of this project is to identify the impact of nsp1 on stress granule formation during SARS-CoV infection. We used human embryonic kidney cells (HEK293) and transfected them with DNA expressing SARS-CoV-1 nsp1 or a control plasmid. Cells were then incubated at 37°C under 5% CO2 concentration for 16 hours. Following incubation, cells were subjected to 30 min of oxidative stress using sodium arenite. Cells were collected and lysed using lysis buffer, then centrifuge at 18 000×g to collect SG pellets used for RNA isolation. Isolated mRNAs were quantified using quantitative RT-PCR. We specifically targeted mRNAs that tend to show a preferential accumulation in SGs without any viral infection. When nsp1 was expressed, we found majority of mRNAs have shown a 2-fold decrease in accumulation in SGs. These results suggest there is a direct effect of nsp1 in dispersing of RNA from SGs. We are currently investigating the effect of viral leader sequence in their accumulation in SGs in the presence of nsp1.

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Abstract 1348

Dynamic RNA acetylation revealed by quantitative cross-evolutionary mapping

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N4-acetylcytidine (ac4C) is an ancient and highly conserved RNA modification that is present on tRNA and rRNA and has recently been investigated in eukaryotic mRNA. However, the distribution, dynamics and functions of cytidine acetylation have yet to be fully elucidated. Here we describe the development of ac4C-seq, a chemical genomic method for the transcriptome-wide quantitative mapping of ac4C at single-nucleotide resolution. In human and yeast mRNAs, ac4C sites are not detected but can be induced – at a conserved sequence motif – via the ectopic overexpression of eukaryotic acetyltransferase complexes. By contrast, cross-evolutionary profiling revealed unprecedented levels of ac4C across hundreds of residues in rRNA, tRNA, non-coding RNA and mRNA from hyperthermophilic archaea. Ac4C is markedly induced in response to increases in temperature, and acetyltransferase-deficient archaeal strains exhibit temperature-dependent growth defects. Visualization of wild-type and acetyltransferase-deficient archaeal ribosomes by cryo-electron microscopy provided structural insights into the temperature-dependent distribution of ac4C and its potential thermoadaptive role. We further describe the appliction of ac4C-seq to study the function of physiological sites of ac4C and the properties of this modification in synthetic mRNAs. Our studies quantitatively define the ac4C landscape and provide a technical and conceptual foundation for elucidating the role of RNA modifications in biology, biotechnology, and disease.

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ASBMB

Abstract 1375

Screening Cas13 guide RNA to evaluate efficiency and specificity

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David Segal, Henriette O'Geen

Today, there are over 7,000 clinically recognized rare disorders, and 80% of these are hereditary. Rare diseases should be prioritized as a public health priority since they frequently affect millions of people worldwide, not just a select few. One of these rare disorders is Angelman Syndrome (AS). It is a hereditary condition caused by the lack of activity of the ubiquitin protein ligase E3A (UBE3A) gene, which is found on chromosome 15. Normally, the UBE3A gene in the brain is solely expressed from the maternal copy. The majority of Angelman syndrome cases involve missing or damaged maternal copies, while the paternal UBE3A allele in neurons is silenced. A protein known as ubiquitin protein ligase E3A, which targets other proteins for cellular degradation, is made with the help of the UBE3A gene. The proper growth and operation of the nervous system depend heavily on ubiquitin protein ligase E3A. UBE3A protein levels in the brain are changed by the paternal UBE3A allele that is silenced in neurons. My research focuses on a gene therapy strategy to reactivate the paternal copy of the UBE3A gene. The paternal UBE3A allele is silenced by a UBE3A antisense transcript (UBE3A-ATS), which silences the paternal allele of UBE3A in cis. This imprinted expression of UBE3A is restricted to neurons. The CRISPR-Cas13d system may theoretically be used to target and cleave the UBE3A-ATS transcript and reactivate the dormant paternal UBE3A gene. My goal is to find out whether a longer gRNA, as opposed to a shorter one, will improve the CRISPR-Cas13 endonuclease target specificity and efficiency. A structure-based gRNA design technique was employed to create gRNAs, with 23 and 30 nucleotides, to the UBE3A-ATS of a rat model of Angelman Syndrome. Efficiencies of different Cas13d gRNAs was evaluated in a synthetic cell model expressing target regions fused to GFP, Rat-1 and Rat-2. Successful cleavage of the Rat-1 and Rat-2 transcripts was assessed by reduction of GFP. Flow cytometry was then used to detect and measure the amount of GFP protein. The data indicate that utilizing the 30-nt gRNA produced equal or better outcomes than the 23-nt gRNA since the 30-nt gRNA had less GFP (activity) than the 23-nt gRNA. As such, my studies suggest that the use of a 30-nt gRNA was better than 23-nt gRNA. The findings are important because they suggest that treating Angelman Syndrome with Cas13d and 30-nt gRNA may be advantageous across different target regions. Future directions in research would evaluate newer forms of RNA-guided and RNA-targeting ribonucleases for increased target specificity and efficiency.

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Abstract 1397

Targeting the Iron Response Element (IRE)-mRNA of Alzheimer's Amyloid Precursor Protein for Binding to Iron Response Protein (IRP1)

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In mRNA a family of 5′-noncoding structures, iron response elements (IRE), control mRNA translation and protein biosynthesis rates by regulatory protein (IRP) binding when iron concentrations decrease IRP protein represses translation by binding to the IRE and inhibiting formation of polysomes. We explored the extent to which the phylogenetically conserved sequence differences among members of the IRE mRNA family related to differences in signal responses observed *in vivo*, since the regulatory proteins are the same for each mRNA. We selected amyloid precursor protein IRE mRNA, to study effects on the 5′ UTR APP mRNA sequence and IRP1 protein binding because of extensive structural information available, and because the increase iron level in the brain related to overexpression of amyloid plaques. We report here the binding affinity of IRP1 protein to APP IRE mRNA. APP mRNA binds to IRP1 with high affinity. Selective destabilization of APP IRE mRNA/IRP1 protein complexes as reported here explain in part the quantitative differences in signal response to iron *in vivo* and indicate possible new regulatory interactions. To understand the relative importance of equilibrium and stability, we further report the thermodynamic of RNA/protein complex. The van't Hoff analysis showed that APP mRNA binding to IRP1 was enthalpy-driven and entropy-favorable. Addition of iron increased the enthalpic contribution by 38% and decreased the entropic influence by 97%. Far-UV CD studies revealed that the APP IRE mRNA formed complex with IRP1 and induced conformational change in the IRP1 protein. The decrease in entropy involved in the formation of the mRNA/protein complex suggested weakened hydrophobic interactions and hydrogen bonding for complex formation and an overall conformational change, and more stable platform for effective APP IRE mRNA/eIFs driven translation.

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Abstract 1417

Cryo-EM structures of the RNA targeting, Cas10-Csm CRISPR system and evidence for a Cas10-activating region within the crRNA-target duplex

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Type III CRISPR systems, exemplified by the Cas10-Csm complex, detect foreign RNAs, such as bacteriophage transcripts, and mount a complex interference response which includes the synthesis of the second messenger molecule, cyclic oligoadenylate (cOA). We determined the structure of *S. epidermidis* Cas10-Csm bound to a target RNA by cryo-EM creating a molecular model for four of the five proteins and both RNAs. The structure reveals how the multi-protein complex grips crRNA and target RNA. Since Cas10 possesses the active site for cOA synthesis, it has been hypothesized that the crRNA-target RNA base pairs located most closely to Cas10 comprise a Cas10-activating region (CAR). COA synthesis by Cas10 is hypothesized to be more sensitive to base pair complementarity within the CAR than to other regions of the crRNA-target duplex. We tested this hypothesis by *in vitro* and *in vivo* assays that reported the effect of mis-pairs on cOA synthesis. These data argue for the existence of a CAR in type III CRISPR. Our structural and biochemical data aids in explaining how interference works in bacteria harboring type III CRISPR systems and will be useful as scientists engineer nucleic acid diagnostics (CRISPR-Dx) that utilize type III CRISPR.

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Abstract 1475

Structural and Functional Characterization of Yeast Splicing Protein Dib1

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The spliceosome is a megadalton ribonucleoprotein complex that catalyzes the removal of non-coding introns and catalyzes the ligation of coding exons. This process is crucial in the transformation of pre-messenger RNA (pre-mRNA) into mature messenger RNA (mRNA) for protein synthesis. At the catalytic core of the splicing machinery lies the 17 kDa protein, Dib1 (*Saccharomyces cerevisiae*), that is present in the pre-catalytic spliceosome but absent in the catalytically active conformation. The loss of Dib1 at this critical transition hints at a potential role in allowing this conformational change to occur. Previous work revealed that Dib1 possesses autocleavage activity, in which Dib1 cleaves the last 14 amino acids from its carboxy-terminal tail. Thus, we are interested in determining whether Dib1's C-terminal tail impacts its function and stability. Previous work found that Dib1 is essential for splicing and cell viability. The temperature-sensitive mutants may suggest that truncations to the C-terminal tail of Dib1 results in a stalled splicing cycle at the B spliceosomal complex. The C-terminal tail may play a direct role in splicing or act as a molecular switch to destabilize Dib1 for departure from the splicing complex. To address these two possible hypotheses, we are investigating the effects of the C-terminal truncations on Dib1 stability and function through growth, splicing assays, and structural assays using circular dichroism spectroscopy. The creation of plasmids with truncations to the C-terminal tail of Dib1 were constructed and transformed into yeast with a dib1Δ for growth assays. Selective plating ensured the retention of the plasmids in transformed yeast. Deletion of fifteen and sixteen amino acids from the C-terminal tail results in an observed widespread growth defect of yeast with a more significant defect at 18°C and 37°C. To explore splicing under these non-permissive temperatures, RNA was extracted from yeast harboring these mutants and grown at non-permissive temperatures and then reverse transcribed. Primers for SUS1 pre-mRNA and mature mRNA were used to monitor splicing of SUS1 in the truncated Dib1 mutants. Truncated proteins were purified, and their stability was assessed. Circular dichroism spectroscopy using increasing concentrations of guanidinium hydrochloride, a protein denaturant, was performed to determine the stability of Dib1 and its truncated mutants. Overall, our findings show that Dib1 tolerates a loss of 5 kJ/mol without affecting cell growth, but additional instability results in faulty splicing and growth

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defects in yeast. Dib1's stability is directly linked to the length of the tail region and leads us to speculate that the tail is critical for stabilization of the spliceosomal complex through protein-protein interactions or protein-snRNA interactions.

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Abstract 1599

The crystal structure of coxsackievirus 5′ RNA replication platform

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The extreme 5′ untranslated region of the CVB3 (+)-sense RNA genome contains a cloverleaf-like (CL) RNA domain that forms an essential platform for viral genome replication. It interacts with the host PCBP and viral 3CD proteins through its stem-loop subdomains sB and sD, respectively. However, atomic-level detail and interactions of the 5′ CL are largely unknown due to the lack of their high-resolution structures. Here, we have reported the crystal structure of intact CVB3 5′CL at 1.9 Å resolution in complex with an antibody chaperone. The crystal structure assumes a compact, H-type antiparallel 4-way junction fold composed of one stem and three stem-loop regions. The subdomains assemble into two sets of co-axially stacked helices, with each coaxial stacking forming almost a continuous A-form helix. The sA helix stacks on the sD helix and the sB helix on the sC helix. Remarkably, the crystal structure also revealed unprecedented long-range interactions between the sC-loop and the sD helix's Py-Py region. These interactions agree well with our NMR results, confirming that these structural features observed in the crystal also exist in the solution. The phylogenetic and 3C protein binding analyses suggest that our crystal structure likely represents a conserved architecture of the enteroviral 5′CLs, including the sC-loop and Py-Py interactions. As our crystal structure represents the first high-resolution structure of the enteroviral 5′CLs, this research will set up a stage for future studies into the mechanism of enteroviral genome replication and the development of antiviral drugs that target this RNA-centric platform.

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Abstract 1679

A Novel Connection Between [PSI+] Prion Formation and RNA Splicing in *Saccharomyces cerevisiae*

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Relatively new studies on prions in *Saccharomyces cerevisiae* show that some protein prion conformations may be beneficial to cell survival in response to environmental stress. Sup35, a translation termination release factor, forms the prion [PSI+]. When [PSI+] forms, translation termination efficiency decreases, which has been shown to result in altered abundance of cellular proteins, including splicing factor abundance. Therefore, we hypothesize that [PSI+] can impact RNA splicing. Indeed, using quantitative RT-PCR we revealed that splicing efficiency for some pre-mRNAs improves upon [PSI+] prion formation. To further test the relationship between RNA splicing and [PSI+] prion formation, we used knockout PCR to create splicing factor deletion mutations in a [psi−] strain, as well as [PSI+] strains. We found that the [PSI+] conformation can suppress the growth defect in a yeast strain with a deletion of the gene encoding MUD2, a protein important for commitment complex formation. In addition, we found that [PSI+] can rescue a splicing defect observed in a *mud2Δ* strain, consistent with the suppressive growth phenotype. Together, the results show that [PSI+] formation impacts RNA splicing in *S. cerevisiae*. Interestingly, stress environments have also been shown to affect splicing in yeast. Based on these data, we hypothesize that [PSI+] formation in *S. cerevisiae* alters RNA splicing to promote cell survival. We are currently monitoring changes in growth and splicing efficiency of [psi−] versus [PSI +], as well as the *mud2Δ* [PSI+], in environmental stresses known to alter splicing, such as LiCl and KCl. Finally, we are completing experiments to determine the mechanism(s) by which formation of [PSI+] alters RNA splicing protein levels. We propose two possible models: readthrough could cause non-stop decay, which would lead to lower splicing protein abundance, or it could lead to nonsense suppression, which could lead to the production of splicing factors with C-terminal extensions and potentially altered functions. Together our studies will illuminate details on a novel role for [PSI+] prion formation in regulating RNA splicing in *S. cerevisiae*, a mechanism that may help yeast survive stress.

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Abstract 1686

Synthetic anti-RNA antibody derivatives for RNA visualization in cells

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Visualization of coding and noncoding RNAs in cells is crucial to understand their roles and functions in various biological processes. Although anti-peptides and anti-protein antibody derivatives such as single-chain variable fragments (scFvs) have revolutionized the imaging and quantification of cellular proteins, there are no antibody-based tools and analogous strategies for RNA imaging. Here, we have developed anti-RNA scFvs and their green fluorescence protein (GFP) fusions as new reagents for RNA visualization in cells. Our approach relies on transforming the existing anti-RNA Fabs into the corresponding scFv formats and generating their scFv-GFP fusions. The scFv probes recognize the target RNA, whereas GFP allows visual detection. As a proof-of-concept, we have developed three scFvs and their scFv-GFP fusions based on the existing BL3-6, HCV2, and HCV3 Fabs. The scFvs have been expressed in BL21 *E. coli* cells and purified using standard chromatographic methods. These recombinantly expressed scFvs, and scFv-GFP fusions bind their cognate RNA targets with affinities similar to their parent Fabs. Further binding studies in cell lysates and structural studies of these scFvs and scFv-RNA complexes are underway. We are also preparing scFv-GFP and target RNA plasmid constructs for mammalian systems to test the feasibility of these antibody tools for live cell RNA visualization. We anticipate that these new tools will bring great opportunities to track RNA molecules and capture their folding dynamics, conformational changes, and localization in living cells.

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Abstract 1722

Deciphering the role of the mitochondrial protein, SUOX, in the nucleolus

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Ribosome biogenesis, which occurs in the nucleolus of eukaryotic cells, is a highly regulated process essential for cell function. Despite its importance and relevance to diseases such as cancer and ribosomopathies, the regulation of ribosome biogenesis in human cells is not fully understood. To identify novel regulators of this process, the Baserga laboratory has pioneered a genome-wide siRNA screen using the number of nucleoli per nucleus as an endpoint. Human MCF10A breast epithelial cells have an average nucleolar number of 2–3, and a decrease to one indicates aberrant ribosome biogenesis. Sulfite oxidase (SUOX) is a mitochondrial protein that has unexpectedly emerged as a hit from this screen. SUOX oxidizes toxic cellular sulfite to sulfate, and mutations are known to cause the severe, fatal developmental disease Isolated Sulfite Oxidase Deficiency (ISOD). Hit validation shows that SUOX depletion reduces nucleolar number and ribosomal RNA (rRNA) biogenesis as measured by 5-ethynyluridine incorporation. We have collected proteomics data upon SUOX depletion that indicates a reduction in essential nucleolar proteins. These data strongly support a role for SUOX in making ribosomes. Lastly, metabolomics analysis from SUOX depleted cells shows a reduction of S'adenosyl-L-methionine (SAM) upon SUOX depletion, hinting at a potential mechanism. SAM is the methyl donor for the nucleolar methyltransferase fibrillarin, which regulates pre-rRNA transcription via H2AQ104Me and pre-rRNA processing via rRNA methylation. The similarity of the presentation of ISOD to that of known ribosomopathies, along with our intriguing preliminary data supporting a role for SUOX in ribosome biogenesis, has raised compelling questions about the involvement of ribosome biogenesis in ISOD pathogenesis. Altogether, our preliminary data strongly support a previously undescribed role for SUOX in ribosome biogenesis with clinical relevance to the disease ISOD. Continued research will define the precise role of SUOX in ribosome biogenesis, including the effects of disease-associated SUOX mutations on this essential cellular process.

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Abstract 1736

RNA interference genes are required for limiting Rad51 protein levels in *Tetrahymena thermophila*

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Connections between endogenous RNA interference (RNAi) pathways and genome integrity have increasingly been reported in diverse species of eukaryotes. Maintaining and protecting genome integrity is an important part of organismal function and health, as evidenced by diseases that result from genome instability, such as cancer. Investigating the genome-protective role that RNAi pathways play in diverse species may lead to a greater understanding of the conserved molecular mechanisms at play. We recently established an RNAi-genome integrity connection in the single-celled eukaryote *Tetrahymena thermophila* through examination of RNAi pathways that depend on three distinct RNA-dependent RNA polymerase complexes (RDRCs) that are required for the biogenesis of ~23–24 nucleotide (nt) small RNAs (sRNAs) (Lee et al. 2021). These sRNAs associate with PIWI protein homologs, called 'TWI' proteins in *T. thermophila*, in sRNA effector complexes. Strains with a knockout of TWI8 or RDN2, a key component of one of the RDRCs, exhibit elevated levels of Rad51, a DNA damage repair protein. However, comparison of the extent of Rad51 elevation between these knockouts and that of a third RNAi gene, RSP1, suggests that additional RDRCs and TWI proteins may also have roles in genome integrity. Our current research aims to more thoroughly elucidate which RDRCs and TWIs in *T. thermophila* are essential for maintaining genome integrity. To do this, we are investigating Rad51 protein levels in an extended set of both single and combination gene KOs of RDRCs and TWIs to comprehensively identify which RNAi genes contribute to genome protection. Preliminary data gathered from this approach supports the importance of more RNAi genes than previously identified in *T. thermophila* genome integrity. Subsets of the combination gene TWI and RDRC KOs exhibited statistically significant Rad51 elevation at the protein level when compared to the unmutated parental, RDN2 KO, and TWI8 KO strains. Our results lay the groundwork for future investigation into how RNAi pathways prevent DNA damage from accumulating in the Tetrahymena genome and thus maintain genome stability. Continued study will provide a deeper understanding of endogenous RNAi pathways, the extent to which their functions and mechanisms are evolutionarily conserved between different eukaryotes, and their significance to cellular and organismal health. Citation: Lee S, Pollard D, Galati N et al. (2021). Disruption of a-23-24 nucleotide small RNA pathway elevates DNA damage responses in *Tetrahymena thermophila*. Mol. Biol. Cell 32, 1335–1346.

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Abstract 1748

Understanding the Effect of Autosomal Dominant Retinitis Pigmentosa Mutations in PRPF6 and PRPF31 on Pre-Messenger RNA Splicing

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The spliceosome is an essential ribonucleoprotein complex that catalyzes the removal of non-coding introns and catalyzes the ligation of coding exons. This process is crucial in the transformation of pre-messenger RNA into mature messenger RNA for protein synthesis. Errors in pre-mRNA splicing are a major cause for human diseases, such as Retinitis Pigmentosa (RP). Retinitis pigmentosa is a degenerative eye disease that causes night blindness, tunnel vision and eventually complete blindness for ~1 in 4000 people. Autosomal dominant Retinitis Pigmentosa (adRP) has been linked to mutations in various proteins in the spliceosome. A majority of these adRP patient derived mutations are found in PRPF6 and PRPF31, two proteins in the U4/U6-U5 triple-snRNP that are required for spliceosome assembly. These mutations may hinder the function of the spliceosome and cause photoreceptor cell degeneration by limiting the necessary spliced mRNA to make critical proteins. The two leading hypotheses for how adRP affects splicing are mutations in essential proteins like PRPF6 and PRPF31 affect retinal mRNA specifically, or they affect splicing throughout the body, but affect retinal genes more severely. To differentiate between these two hypotheses, we are exploring the ramifications of these mutations on cell growth and splicing using a mouse photoreceptor cell (661W). We have created stably transfected cell lines with adRP mutations PRPF6 and PRPF31 and have examined the cell lines for changes to cell morphology and effects on splicing. Western Blot and immunofluorescence have confirmed the expression of mutant protein. Our recent findings show a clear change in cell morphology for several adRP mutants.

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Abstract 1776

Orphan Riboswitch Candidates Sense Fundamental Metabolites

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Riboswitches are structured non-coding RNA elements typically found in the 5′ untranslated regions (UTRs) of bacterial mRNAs, where they regulate gene expression most commonly at the level of transcription or translation. Each riboswitch employs a conserved aptamer domain that selectively binds a ligand, such as a metabolite or elemental ion, to induce a conformational change in a downstream expression platform. More than 55 unique riboswitch classes have been experimentally validated to date. However, the cognate ligands of many of the more than 100 'orphan' riboswitch classes remain unknown, mostly because clues regarding the identities of their ligands are lacking. We have embarked on an analysis of several orphan riboswitch candidates to identify their ligands and validate their regulatory mechanisms. To achieve these objectives, we are using a series of bioinformatic, biochemical, and genetic approaches that reveal functional details regarding these RNAs. Herein we present our progress regarding several orphan riboswitch candidates previously discovered by comparative sequence analysis. One of these riboswitch candidates is the algC motif that is found upstream of the cpsG gene This gene codes for a mannose-6-phosphate isomerase relevant to colanic acid biosynthesis, which is the major component of the protective capsule encompassing certain bacterial cells. Experimental evidence has established the algC motif functions as a genetic switch. Furthermore, a transposon-mediated knockout screen in *Escherichia coli* uncovered genes relevant to mannose metabolism whose disruption cause dysregulation of a reporter gene fused to an algC motif RNA representative. Further efforts to discover the cognate ligand for the algC motif are underway, and to establish the contribution of this riboswitch candidate to sugar metabolism, colanic acid biosynthesis, and other possible biological pathways in bacteria.

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Abstract 1806

Star-PAP Controls Oncogene Expression and Tumorigenesis Through Primary miRNA processing in Breast Cancer

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Pre-mRNA 3′-end processing is a key post-transcriptional event that controls the expression of both oncogenes and tumor suppressors. Studies have shown that the non-canonical poly(A) polymerase, Star-PAP regulates 3′-end polyadenylation of mRNAs encoding anti-invasive factors in breast cancer. In addition, Star PAP expression is reduced in cancer and acts as a tumor suppressor protein. Yet, the mechanism how Star-PAP controls various cellular processes in cancer progression are still unclear. To understand this, we employed Star-PAP RNA binding mutant (S6A) that is compromised for RNA 3′-end formation and analyzed in a xenograft mice model. We observed a reduction in the tumor formation on Star-PAP ectopic expression in MDA-MB231 cells (where Star-PAP expression is negligible) in the xenograft model that is ameliorated by Star-PAP S6A mutant. Concomitantly, we observed a reduced cell proliferation in MDA-MB231 cells on Star-PAP expression but not on S6A mutant expression. Similarly, in MCF7 cells (high Star-PAP expression), Star-PAP depletion induces cellular proliferation and that is reduced on wild-type expression but not by S6A Star-PAP. To understand the molecular mechanism, we analyzed Star-PAP global mRNA profile after Star-PAP knockdown that shows a large fraction of targets that are primarily up regulated (pro-tumor) in cancer. Interestingly, majority of these mRNAs are independent of alternative polyadenylation (APA)-mediated UTR regulation indicating a distinct mechanism of Star-PAP control of oncogene expression. We have confirmed induced expression of select target oncogenes (CDK6, ATG9A, MTA1, KDM5A and MEF2D) on Star-PAP knockdown that is rescued by WT but not but S6A Star-PAP in both the cancer cell lines. We demonstrated that Star-PAP regulates target oncogenes through control of cellular miRNA levels that are down regulated in cancer. Induced target oncogene expression and concomitant cellular proliferation on Star-PAP knockdown was rescued by introduction of mimics of the targeted miRNA (miR421 and 424), whereas Star-PAP depletion did not show any discernible effects in the presence of miRNA inhibitors in MCF7 cells. Similarly, in the MDA-MB231 cells, ectopic Star-PAP expression reduced both target oncogene expression and cellular proliferation that was rescued by miRNA inhibitor while Star-PAP expression did not have any affect in the presence miRNA mimics. Further, we have confirmed loss of miRNA expression (miR 421, 424, 543, 335, 26A) that is rescuable on Star-PAP ectopic expression in MCF7 cells. Interestingly, cellular miRNA levels in both MCF7 and MDAB-231 cells were directly correlated with the difference in the cellular Star-PAP levels in the two cell lines. Further, to understand how Star-PAP regulates miRNA expression, we analyzed steps of miRNA biogenesis that includes generation of primary miRNA (pri-miRNA), precursor miRNA (pre-miRNA), export to the cytoplasm, and mature miRNA generation and its stability. We demonstrated that Star-PAP specifically regulates processing and synthesis of Pri-miRNA transcript thus controlling both pre-miRNA and mature miRNA generation. Our study established a novel mechanism of oncogene regulation through Star-PAP mediated pri-miRNA processing that controls both cellular proliferation and tumorigenesis.

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Abstract 1814

The Autocleavage Ability of Human Spliceosomal Protein Dim1

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Pre-messenger RNA splicing is mediated by the spliceosome, a dynamic macromolecular complex composed of small nuclear ribonucleoproteins (snRNPs) and other associated proteins. At the catalytic center of this complex is the human protein Dim1. hDim1 is a 142 amino acid protein with a thioredoxin-like fold that is essential for cell viability. Within the splicing cycle, hDim1 departs the spliceosome in its transition from a pre-catalytic to a catalytic conformation. Its departure within this highly regulated transition leads to the hypothesis that hDim1 serves as a linchpin in the splicing cycle to prevent splicing from occurring prematurely. Interestingly, hDim1 has demonstrated peptidase activity, specifically autocleavage of its own carboxy-terminal tail. Autocleavage of hDim1 results in the truncated form of the protein hDim1ΔC14 with its flexible tail released. Autocleavage has previously been determined to be dependent on divalent metal ions and has been found to be inhibited by peptidase inhibitors. The mechanism and function of hDim1 autocleavage remains highly uncharacterized; therefore, we are interested in investigating this reaction. Purified recombinant hDim1 was utilized to study *in vitro* autocleavage reaction in the presence of zinc metal ions to determine under what conditions this reaction occurs best. The thioredoxin-like core of hDim1 suggests that cysteine residues are important for this catalytic function, although no specific function has been attributed to these residues. To examine the importance of these residues in autocleavage, we are examining thiol-reducing conditions in conjunction with the other reaction conditions. Optimizing the conditions under which autocleavage occurs may implicate the residues responsible for the catalytic activity of hDim1 and will provide insight into the mechanism of this reaction.

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Abstract 1823

Biochemical and Genetic Evidence Support Fyv6 as a Splicing Factor Involved in Exon Ligation

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Removal of introns and ligation of exons via splicing of pre-mRNAs are essential processes for the regulation of gene expression in all eukaryotes. Splicing is carried out by the spliceosome, a large and highly dynamic complex, comprised of 5 small nuclear ribonucleoproteins (snRNPs) and dozens of additional proteins. Mutations within the spliceosome are responsible for various diseases in humans caused by disruption of splicing regulation. Therefore, identifying splicing factors and analyzing the interactions between them is essential for understanding human gene expression and health. Through cryo-electron microscopy (cryoEM), human protein FAM192A was recently identified as a new splicing factor present in catalytic spliceosomes and Fyv6 was predicted to be the FAM192A homolog in *S. cerevisiae* (Zhan, X. et al., Mol Cell 2022). However, no biochemical evidence was provided to confirm Fyv6's role or determine if it was essential for splicing in yeast. Herein, we show that the *S. cerevisiae* homolog, Fyv6, is a splicing factor and likely a component of the catalytic yeast spliceosome. We first constructed Fyv6 knockout strains to use in our studies. Yeast lacking Fyv6 are viable but have growth defects indicating that the protein is not essential for splicing but could play a role in the process. We then assayed for genetic interactions between Fyv6 and well-studied splicing factors Prp8, Prp16, and Prp22. We found strong genetic interactions between Prp8 and Fyv6 with Prp8 alleles defective in the 2nd step of splicing being synthetically lethal with Fyv6 deletion and Prp8 alleles defective in the 1st step of splicing suppressing a Fyv6 deletion phenotype. We then used ACT1-CUP1 assays to directly measure splicing efficiency *in vivo*. These results revealed that Fyv6 knockout strains exhibited large deficiencies in splicing of introns with certain nonconsensus splice site and branch site sequences. Overall, our results confirm that Fyv6 does play a role as a splicing factor and provides biochemical and genetic evidence supporting the protein as a component of the yeast spliceosome.

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Abstract 1828

Divergent Roles of Dicer's Helicase Domain in Antiviral Defense

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In vertebrates, viral dsRNA is recognized by RIG-I-like receptors (RLRs) to trigger an interferon response, while in invertebrates, Dicer cleaves viral dsRNA to generate siRNAs that silence viral transcripts. RLRs and Dicer all contain a conserved helicase domain, suggesting this domain functioned in antiviral defense in a common ancestor. Our comparative studies of invertebrate and vertebrate Dicers reveal interesting differences, likely reflecting divergence of innate immune pathways. Our cryo-EM and biochemistry analyses of invertebrate *D. melanogaster* Dicer-2 show that its ATP-dependent helicase domain recognizes ends of viral dsRNA, initiating threading through the helicase domain, and processive cleavage by the RNase III domains. By contrast, *H. sapiens* Dicer cannot hydrolyze ATP, and it is controversial whether this enzyme functions in antiviral defense. We used ancestral protein reconstruction to elucidate the evolutionary history of Dicer's helicase domain in animals. We predicted protein sequences of common ancestor helicase domains, and biochemically expressed and characterized their recombinant forms. Our studies indicate ancestral animal Dicers possessed a functional helicase domain that coupled dsRNA binding to ATP hydrolysis. However, at the common ancestor of jawed vertebrates, coinciding with appearance of interferon-based innate immunity, helicase function was lost, and dsRNA binding was impaired. Invertebrates lack an interferon pathway, and correspondingly, many lack the RLRs that recognize viral dsRNA in vertebrates. However, in *C. elegans*, viral dsRNA is processed by a complex containing *C. elegans* Dicer (DCR-1), the dsRNA binding protein RDE-4, and an ortholog of mammalian RIG-I called DRH-1. We biochemically characterized the *C. elegans* antiviral complex. To correlate activities with specific proteins, we compared the wildtype complex to complexes lacking RDE-4, or with point mutations in the Walker A motif of the helicase domain of DCR-1 or DRH-1. Cleavage, binding, and ATP hydrolysis assays indicate RDE-4 is important for dsRNA binding, and that both helicases have ATP hydrolysis functions. Interestingly, our analyses indicate DRH-1 has a more prominent role in ATP hydrolysis than DCR-1; our recent cryo-EM analysis of the complex provides important context for the activities of each protein. Going from flies to worms to humans, our studies highlight the journey of ATP-dependent innate immunity as it became less dependent on Dicer, and more reliant on the RLR proteins that dominate mammalian antiviral defense.

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Abstract 1882

RNA exosome cofactors role in the variability of outcomes when mutations occur in the RNA exosome complex

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The RNA exosome is an evolutionary conserved ten subunit protein complex that processes and degrades RNA in all cell types. The essential RNA exosome complex is composed of a three-subunit cap and a six-subunit ring central ring with a catalytic 3′–5′ exonuclease subunit at the base. The RNA exosome plays an important role in degrading improperly processed RNA. The structural units of the complex serve as a tunnel so that the substrate RNA can be channeled to the catalytic subunit DIS3/Rrp44 for degradation. As the RNA exosome both processes and degrades, many different RNAs, cofactors that associate with the complex, help guide and direct specific RNAs towards this ring structure as well as remodel RNA substrates to facilitate degradation. Missense mutations in genes that encode structural subunits of the RNA exosome are associated with a multitude of diseases not limited to, but including retinitis pigmentosa, pontocerebellar hypoplasia, hypothyroidism, neurodegenerative diseases, and muscle atrophy. How mutations in genes that encode an essential complex required for fundamental cellular functions affects some body systems and cell types more severely is not yet understood. One model under investigation is that different cells may have different RNA exosome cofactors, such as the helicase Mtr4 and the scaffolding SKI complex, which need to associate with the complex to target specific RNAs. Finally, we will use the 3-dimensional chemical viewing program JMOL and 3D printing to represent the overall structure, conformational changes, and proposed interactions of the RNA exosome subunits.

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Abstract 1890

Analyzing the role of RNA interference in limiting micronuclei-like bodies in *Tetrahymena thermophila*

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RNA interference (RNAi) pathways have emerged as processes of interest potentially involved in the maintenance of genome integrity across a variety of eukaryotes, including humans. Previous research done on *Tetrahymena thermophila* has shown that one of the predominant roles of endogenous RNAi pathways in this organism during growth is to maintain genome integrity. In *T. thermophila*, this pathway depends at least in part on Rdn2, a critical component of one of three known RNA-dependent RNA polymerase complexes (RDRCs) which act as processing factors for distinct types of small (s) RNA precursor transcripts. RDRCs process sRNA precursors into double stranded RNA, which is diced into ∼23–24 nucleotide sRNAs. These sRNAs then interact with a subset of PIWI homologs called "Twis" in *T. thermophila*. Notably, RDN2 knockouts exhibited enlarged chromatin extrusion bodies (CEBs), which are extranuclear DNA-containing structures in *T. thermophila* that are thought to be analogous to micronuclei often observed in human cancer cells. The presence of these structures is correlated with changes in chromatin structure and/or increased DNA damage in *T. thermophila*. However, the contribution of Twi proteins and the two other RDRCs on CEB size and accumulation was unknown. To investigate this, we examined the number and size of chromatin extrusion bodies (CEBs) in single and combination TWI and RDRC gene knockout strains, focusing on genes previously linked to the ∼23–24 nt sRNA pathways. To efficiently and reproducibly quantify CEB areas, we developed a semi-automated approach using FIJI/ImageJ. Our study has revealed that a subset of Twis is fundamental in limiting CEB size and suggests that more than one Twi may work together to maintain genome integrity. Our findings provide a promising foundation for future studies into the mechanisms of RNAi pathways in maintaining genome integrity in *T. thermophila*, which may ultimately provide insights that are generalizable to other eukaryotes.

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Abstract 1896

Regulation of senescent cell survival by a GBA pseudogene

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Selective clearance of senescent cells *in vivo* has been shown to delay emergence of aging-associated diseases and to lengthen lifespan. The metabolic processes occurring within senescent cells are key to their special properties. Here, we used CRISPR-based screening to identify and characterize a long noncoding RNAs (lncRNAs) that support survival of senescent cells through metabolic regulation. In order to study survival of senescent cells, we used the DNA damage response of BJ fibroblasts paradigm and conducted a CRISPRi-based loss-of-function screen targeting all lncRNAs expressed before and after cells enter senescence. Guides depleted in cells 10 days after senescent entry were used to identify lncRNAs required for senescent cell viability. Candidates were validated and followed up using siRNA-/sgRNA-mediated knockdown followed by RNA sequencing and study of cell biology and lipidomic profiling. We identified two lncRNAs LINC01503 and GBAP1, a pseudogene of GBA, as required for survival of senescent cells, as cell numbers decreased upon their knockdown before or after DNA damage. LINC01503 loss affected expression of adjacent genes, and so it appears in act in cis-acting regulation of gene expression. Loss of GBAP1, an unprocessed pseudogene transcribed from a genomic locus proximal to GBA1, lead to reduced GBA activity and led to a dramatic increase in the levels of its substrates, the glucosylceramides. Surprisingly, knowdown of GBAP1 did not markedly affect GBA mRNA or protein levels, but rather specifically led to a decrease in accumulation of the glycosylated form of GBA. RNA-seq data and immunofluorescent staining indicates this phenotype likely stems from reduced processing of GBA in the endoplasmic reticulum. We thus describe a new functional role for a pseudogene lncRNA in regulating the post-translational processing of the product of its parent gene. More broadly, the tissue- and cell type-specificity of lncRNA expression raises hope that targeting such lncRNAs with a role in senescence may result in clearance/change of behavior of these cells *in vivo* with limited side-effects throughout the body.

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Abstract 1915

Overturning the Paradigm of miRNA Regulation: Upregulation as a common mechanism of miRNA action

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microRNAs (miRNAs, miRs) are small non-coding RNA that tune protein expression through modulation of mRNA. The canonical view of miRNAs is that they are posttranscriptional repressors, binding to the 3′-untranslated region (UTR) of mRNA and causing mRNA destabilization and/or loss of translation. Using our recently developed high-throughput fluorescence assay (miRFluR), we have comprehensively mapped the miRNA regulatory landscape of multiple glycosylation enzymes and found, contrary to expectations, miRNA predominantly upregulate protein expression of some messages (e.g. ST6GAL1). In contrast, for other mRNAs (ST6GAL2 and B3GLCT) the role of miRNA were predominantly down-regulatory, although some upregulatory interactions were observed. Mutational analysis identified direct binding sites in the 3′-UTR responsible for upregulation, confirming it is a direct effect. The miRNA binding proteins AGO2 and FXR1 were required for upregulation. Our results upend common assumptions surrounding miRNA, arguing that upregulation by these non-coding RNA is common. In this presentation, we will discuss our latest work dissecting upregulation and the role of the 3′-UTR.

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Abstract 1921

DNA damage signaling and repair is elevated upon disruption of an endogenous RNA interference pathway

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There is a growing body of knowledge linking RNA interference (RNAi) to protection of the genome in many eukaryotes. Previous research identified a novel RNAi pathway in the single-celled eukaryote *Tetrahymena thermophila* that produces small RNAs (sRNAs) that are ~23–24 nucleotides (nt) in length. Recently published work showed this RNAi pathway to be critical for the genome, as cells lacking specific RNAi factors had elevated levels of Rad51, a DNA repair protein, and gamma-H2A.X, a marker of double-stranded DNA breaks. The study of genome protective mechanisms such as those involving RNAi factors and sRNAs is important, given that the dysregulation of DNA integrity maintenance can lead to human disease, such as cancers. The ~23–24 nt sRNA RNAi pathway in *T. thermophila* produces sRNAs that are predominantly derived from putative precursor RNAs expressed from four types of genomic locations. Biogenesis of three of these sRNA classes are dependent on one or more RNA-dependent RNA polymerase complexes (RDRCs). Once produced, the sRNAs interact with a class of PIWI-homolog effector proteins known as "Twis" in *T. thermophila*. The molecular functions of the resulting sRNA-Twi complexes are currently unknown. Recent work from our lab demonstrated that one RDRC factor responsible for two distinct sRNA classes, Rdn2, and a Twi with nuclear localization, Twi8, are required for limiting the protein levels of the DNA repair protein Rad51. However, comparison of the Rad51 phenotype in RDN2 and TWI8 knockouts to each other and to a knockout of a third gene, RSP1, which disrupts all RDRC-dependent RNAi pathways, suggested that additional RDRCs and/or Twis may be involved in genome integrity maintenance. Therefore, to gain a more comprehensive view of the RNAi factors involved in this genome integrity function in *T. thermophila*, we produced and analyzed a variety of single gene and combination gene knockouts and examined nuclear Rad51 and gamma-H2A.X levels. Our preliminary data indicates that RSP1 may contribute to genome integrity in a way that is distinct from the RDRCs themselves. In addition, more than one Twi is important for protecting the genome from accumulated DNA damage. These findings offer important insights that will enable further research into the mechanisms by which *T. thermophila* RNAi machinery protects the genome. This work also has implications for a broader understanding of RNAi and its link to genome integrity, given the conservation of this link in many branches of the eukaryotic evolutionary tree.

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Abstract 2000

Circular RNAs expressed in brain and associated with Alzheimer's Disease are translated

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The molecular changes leading to Alzheimer's disease (AD) progression are poorly understood. A decisive factor in the disease occurs when neurofibrillary tangles composed of microtubule associated protein tau (MAPT) form in the entorhinal cortex and then spread throughout the brain. Our objective was to identify molecular mechanisms that drive Alzheimer's disease progression. We concentrated on two new forms of RNA, circular RNAs (circRNAs) and regulated retained introns. Both RNAs are changed during AD progression and importantly, we found that circRNAs are translated after undergoing adenosine to inosine RNA editing, possibly leading to an Alzheimer's disease specific proteome.

Methods: Total RNA was isolated from human brain (entorhinal and frontotemporal cortex). Poly(A)+ RNA was subjected to Nanopore sequencing. Total RNA was analyzed by standard Illumina sequencing. Circular RNAs were sequenced from RNase R treated and rRNA depleted total RNA. The sequences were analyzed using Bowtie2 (v2.2.3), Samtools (v1.11) mpileup, and custom pileup2base scripts. Expression constructs for circRNAs were cloned into pcDNA3.1 with a start codon downstream of a 3x FLAG tag or without a start codon. The constructs were co-transfected with ADAR1-3 expression constructs in HEK293 cells to evaluate protein expression using Western Blot and the new proteins were validated using Mass Spectrometry.

Results: We identified numerous circRNAs from which 453 correlates with Alzheimer's disease severity, expressed as Braak stages. For all circRNAs, there is a statistically significant increase and correlation of adenosine to inosine RNA editing with Braak stages. Importantly, this correlation cannot be detected with mRNAs. We thus cotransfected highly expressed circRNAs correlating with Braak stages with ADAR1 and observed RNA editing-dependent translation for circMAN2A1, circHOMER1 and circNOGO. These data confirm our report that adenosine to inosine RNA editing promotes circRNA translation, which we reported for the circMAPT system. Further, Nanopore sequence identified for the first time regulated retained introns in human polyadenylated mRNAs that likely allow rapid responses to neuronal stimuli.

Conclusions: Our data indicate that the expression of several circRNAs correlate with AD progression in entorhinal cortex. There is a general increase in A > I editing during Alzheimer's disease progression which likely leads to circRNA translation. Thus, circRNAs can generate new Alzheimer-specific peptides by using reading frames and/or start codons in the circular RNAs that are different from linear RNAs. Due to rolling circle translation, multimers can be formed in the absence of stop codons, as exemplified for MAPT. This new pathomechanism creates a new 'circRNA-specific' proteome in Alzheimer's Disease which in case of circMAPT contributes to Alzheimer's disease by forming Neurofibrillary Tangles.

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Abstract 2050

Structural characterization of the HERV-K Rec protein

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Human Endogenous Retrovirus-K (HERV-K) is the most recently acquired retrovirus in the human genome, and therefore it is the most actively expressed as it has accumulated fewer inactivating mutations over time. HERVs are expressed upon exposure to external physical (e.g. irradiation) and chemical agents, as well as viral infections. The HERV-K Rec protein is a nuclear transport protein that is required for viral gene expression. Given that HERV-K is known to be involved in several cancers and neurodegenerative diseases, the Rec protein, itself an oncoprotein, may offer a target for therapeutics once its structure is elucidated. Unlike the similar Rev protein of HIV, the structure of the Rec protein has not been fully characterized. Our objective is to purify the Rec protein in order to crystallize and characterize its three-dimensional structure. Rec mostly accumulates in the insoluble fraction at all attempted over-expression conditions including 0.1 mM or 1 mM IPTG; 37°C, 22°C, or 16°C incubation;, and 4–24 hour expression. In order to solubilize the protein, a 1% SDS-containing buffer was used. Following solubilization, the His-tagged Rec protein was purified on a nickel-NTA column and eluted with imidazole. Following purification, the protein was dialyzed or diluted dropwise in a phosphate-based buffer to refold the protein. A precipitate formed during dialysis, so the sample was re-solubilized with urea, then diluted dropwise in a HEPES-based buffer. This treatment resulted in a soluble protein. Protein-RNA assays are in progress to assess the functionality of the refolded protein.

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Abstract 2060

Optimization of protein expression and purification of R2Bm mutant proteins

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Long Interspersed Elements (LINEs) are retrotransposons found in most eukaryotic genomes and are among the most abundant transposable elements found in the human genome, accounting for over one third of our DNA. While most of LINEs found in the human genome are inactive, active LINEs are occasionally responsible for diseases including some types of cancer, hemophilia and muscular dystrophy. Study of LINEs is invaluable, not only for their role in clinical implications but also for evolutionary tracking and for more in-depth understanding of our own genome. In addition, it can provide insight into how other retrotransposons with similar mechanisms integrate into host genomes and how retrotransposons could be modified for gene delivery purposes in the future. Transposition of LINEs begins by DNA cleavage activity of an element encoded endonuclease, which releases a 3′-OH group. Element encoded reverse transcriptase then uses the 3′-OH and directly reverse transcribes the element RNA into cDNA at the target site. This is known as Target Primed Reverse Transcription (TPRT). R2 elements from Bombyx mori (R2Bm) belong to an earlier branching group of LINEs with a single Open Reading Frame (ORF) consisting of Myb and zinc finger (ZF) motifs, a region implicated in RNA binding (-1), a reverse transcriptase (RT), a cysteine-histidine-motif (CCHC), and a type II restriction-like endonuclease (RLE). These elements exclusively insert into a fraction of the multi-copy 28S rRNA genes in a variety of animal taxa. In this study, site directed mutagenesis was used to mutate highly conserved regions within the Reverse Transcriptase and Thumb region of R2Bm protein. These conserved regions are hypothesized to be involved in nucleic acid binding which in turn is critical for the TPRT mechanism. Double alanine substitutions (e.g. HRKK to HRAA, RK to AA and KPQ to APA) were made directly in the His-tagged wild type expression vector and the mutant constructs were transformed into BL21 cells and plated on LB with Kanamycin for selection. Single Kanamycin resistant colonies from each of the mutants were grown in LB with Kanamycin media and mutant R2Bm protein expression was induced using Isopropyl β-D-1-thiogalactopyranoside (IPTG). Mutant protein purification was optimized using affinity (Talon resin)

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chromatography. Purified wild type and mutant proteins were run on SDS-PAGE gels and the amounts of purified proteins were quantified by using a Bovine Serum Albumin (BSA) standard curve. Based on our results, we conclude that we were able to successfully optimize the expression and purification of the mutant proteins and the amounts of mutant proteins purified were similar to that of the wild type R2Bm protein.

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Abstract 2075

Thermodynamic Analysis of 1 × 2 Internal Loop Regions of SL1 of SARS-CoV-2 and SL of U6 snRNA

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The genomic material of SARS-CoV-2 is a positive-sense single-stranded RNA. SARS-CoV-2 produces non-structural protein 1 (NSP1), which inhibits host cell translation by binding its' N-terminal to the host's 40S ribosomal subunit. Once NSP1 is bound its C-terminal domain folds and binds to the mRNA entry channel. Stem loop 1 (SL1) in the 5′-UTR of the viral mRNA binds to NSP1 to abrogate translation inhibition leading to the expression of viral proteins. SL1 contains a 1 × 2 internal loop that is not seen in other coronaviruses and may be involved in conformational changes that influence SL1-NSP1 interactions. The 1 × 2 internal loop of SL1 contains a putative A•C non-canonical base pair. The U6 snRNA also contains a 1 × 2 internal loop known to undergo conformation changes in response to pH and magnesium ion binding. Here we examine the thermodynamic properties and magnesium binding of the 1 × 2 internal loop of SL1 in varying helical contexts. Thermal denaturation experiments were performed on various DNA and RNA constructs in the presence of 1 M KCl or 10 mM magnesium chloride at a pH of 5.5 and 7. We show that formation of the A+•C base pair and the construct stability in the presence of magnesium ions is dependent on the helical context.

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Abstract 2080

Modeling DNA Oligonucleotide Binding to DNA-mimetic Stem-Loop 1 of the SARS-CoV-2 RNA

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Neena Grover

SARS-CoV-2 is a positive-sense RNA virus that contains open reading frame 1ab (ORF1ab) to produce 16 nonstructural proteins (nsps). Five stem-loops (SL) are found in the 5′ UTR of the RNA that are involved in myriad viral functions and are labeled SL1 through SL5. SL1 is crucial to viral replication. Upon viral infection, nsp1 binds the ribosomal 40S subunit to inhibit all host mRNA translation. Upon SL1 binding to nsp1, viral mRNA can be processed by the ribosome, allowing viral proteins to be produced. In this study, we are examining small DNA oligonucleotides that bind to SL1-mimetic DNA in order to block SL1-nsp1 interactions. We designed a DNA analog of the SL1 hairpin and two small DNA oligonucleotides that are complementary to either the helical stem or the loop region of SL1. The binding of these oligonucleotides to the SL1 hairpin should allow the formation of either an alternate duplex or a triplex structure. Isothermal titration calorimetry (ITC) and circular dichroism (CD) techniques were performed in 1 M KCl and 10 mM MgCl2 at two different pH (5.5 and 7.0) to examine structural and thermodynamics of binding. ITC of the two oligonucleotides showed modest binding. Results from DNA binding experiments, thermal denaturation, and CD show the hairpin structure is thermodynamically more favored and mostly remains intact under the conditions examined.

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Abstract 2083

A single tyrosine in a PUF RNA binding protein controls its mRNA binding landscape and separates its distinct biological functions

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PUF (Pumilio and FBF) RNA-binding proteins (RBPs) control a range of biological functions, including stem cell maintenance. Moreover, dysfunction of its human PUM ortholog has clinical consequences, including neuronal disorders, infertility and cancer. We sought to understand how PUF proteins elicit distinct biological outcomes, even within a single tissue. *C. elegans* FBF-2 is a paradigmatic metazoan PUF responsible for two distinct functions in the germline: stem cell maintenance and oocyte fate specification. *In vitro*, a single FBF-2 tyrosine mediates FBF-2 binding to various protein partners, and we wondered if that residue distinguishes its two biological roles. To test this, we mutated the tyrosine to alanine in endogenous fbf-2 using CRISPR-Cas9 mediated genome editing. Remarkably, FBF-2 Y479A retains its ability to maintain stem cells but loses its ability to specify the oocyte fate. Thus, this single tyrosine is critical for one FBF-2 function but not the other. FBF-2 Y479A is expressed normally and has the same intrinsic regulatory activity as wild-type FBF-2. We therefore asked if this residue change alters the FBF-2 RNA binding landscape using enhanced crosslinking and immunoprecipitation (eCLIP). Wild-type FBF-2 binds ∼1700 mRNAs, FBF-2 Y479A binds 85% of those mRNAs to a similar extent and at the same sites. However, the remaining 15% fall into two classes. FBF-2 Y479A associates with some mRNAs more strongly than wild-type and others more weakly. We are currently testing representative RNAs of each class for changes in expression of tagged fluorescent proteins using immunocytochemistry. Wild-type FBF-2 and FBF-2 Y479A bind the daz-1 3′UTR similarly and DAZ-1 expression appears unchanged in the mutant. By contrast, wild-type FBF-2 has a major peak in the gld-1 3′UTR, that is reduced 4-fold in FBF-2 Y479A, and expression of the GLD-1 protein is significantly more abundant in the mutant. At the other end of the spectrum, wild-type FBF-2 has no peak in the ife-3 coding region but FBF-2 Y479A has a significant peak there and expression of the IFE-3 protein is less abundant in the mutant. Therefore, FBF-2 Y479A can change where the protein binds within RNAs and either increase or decrease their expression. In sum, a conserved protein-protein interface can affect one fate decision by changing the regulation of a few specific mRNAs among the many bound. Since protein partnerships are central to the control of many mRNAs, these principles likely apply to other RBP networks as well.

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Abstract 2086

A bacterial auxotroph system for novel riboswitch selection

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Riboswitches are metabolite-sensitive regulatory elements found in the 5′-leader sequences of many bacterial mRNAs. A significant goal in synthetic biology is to develop new riboswitches that have potential as useful mechanisms for regulation of RNA therapeutics and biosensing applications. Development of novel practical small molecule binding elements called "aptamers" capable of efficiently communicating with RNA regulatory switches has remained a challenge in accomplishing this aim. As one approach to address this, we are repurposing a bacterial double auxotroph selection system initially employed for transcription factor binding specificity determination (Meng and Wolfe, Nat. Protocols 2006) to identify novel riboswitches responsive to alternate ligands from large libraries. A riboswitch scaffold library containing randomized aptamer sequences influences positive and negative selectable markers, HISB and URA3 respectively in a bicistronic reporter plasmid. In medium without histidine, members possessing a riboswitch responsive to ligand are conferred a survival advantage by reduced attenuation in expression of HISB, an essential enzyme in histidine biosynthesis. Initial validation experiments using a known adenine-responsive riboswitch derivative inserted upstream of the reporter genes demonstrate a >6-fold increase in growth after 18 hours when treated with 2-amino-purine, verifying survival advantage conferred by functional sequences. Furthermore, selection in solid drop-out media yields effective elimination of cells containing plasmids with undesired sequences attenuating expression even in the presence of purine ligand, confirming selection against these non-functional members. Counterselection method development progress indicates potential to effectively eliminate evasive constructs to isolate functional riboswitches of interest. Successful implementation of this approach will yield a selection tool for accelerating discovery of RNA regulators responsive to novel small molecules.

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Abstract 2090

Probing Non-Canonical A•C Base Pairs in DNA using SERS

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Raman spectroscopy probes the vibrational modes of a molecule. In recent years, surface-enhanced Raman spectra (SERS) of oligonucleotides on gold or silver nanoparticles have yielded significantly stronger signals. Raman spectra of DNA are high throughput, quantitative, and label-free and show distinct features created by vibrational modes such as ring deformation, backbone bending, and hydrogen bond stretching. Here we are using gold nanoparticles to probe various structural changes in a short helical DNA designed to mimic SL1 of coronavirus and SL of U6 snRNA. Phosphate buffers containing 1 M KCl or 10 mM magnesium chloride were utilized at two different pH (5.5 and 7). Differences in peak intensity are being observed between canonically paired helical DNA and DNA of similar composition with modifications containing non-canonical A•C base pair. We are comparing ion binding, pH-related, and temperature-variable conditions to observe changes in DNA structures.

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Abstract 2098

Role of *Trypanosoma brucei* prolyl-tRNA synthetase and a trans-editing domain in maintaining translational fidelity

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Aminoacyl-tRNA synthetases (ARSs) catalyze a two-step aminoacylation reaction wherein tRNAs are charged with specific amino acids. Some ARSs misacylate structurally similar amino acids onto their cognate tRNAs, and organisms across all domains of life have evolved proofreading strategies to ensure high fidelity of protein synthesis. *Trypanosoma brucei* (Tb) is a eukaryotic pathogen that causes Human African Trypanosomiasis (HAT)—a fatal disease that currently lacks reliable therapeutics. Before infecting mammals, Tb resides in the midgut of the tsetse fly. Differentiation of Tb from the insect form to the mammalian bloodstream form leads to metabolic reprogramming. In a nutrient-rich mammalian host environment, Tb uses glucose for ATP production via glycolysis. In the insect form, glucose availability is low and proline is imported as a carbon source to make ATP, producing alanine as a major side product. Thus, tRNAPro aminoacylation errors may be elevated during insect-stage catabolism, as prolyl-tRNA synthetases (ProRSs) across all domains of life mischarge alanine onto cognate tRNAPro. While most bacteria encode a ProRS containing an Ala-tRNAPro editing domain (INS) inserted within the catalytic core, eukaryotic ProRSs lack an INS domain but often encode a freestanding Ala-tRNAPro trans-editing INS-domain homolog, ProXp-ala. In contrast, Tb encodes a putative ProXp-ala editing domain that is appended to the N-terminus of ProRS, along with a previously uncharacterized free-standing ProXp-ala-like domain, MCP3. We hypothesize that both putative editing domain homologs are responsible for ensuring translational fidelity in Tb. To test this hypothesis, we recombinantly expressed and purified full-length Tb ProRS, the ProXp-ala domain of ProRS alone, and MCP3 and performed *in vitro* kinetic assays. The full-length enzyme deacylated Ala-tRNAPro ∼17.0-fold faster than the freestanding ProXp-ala domain. Surprisingly, MCP3 is an even more robust Ala-tRNAPro deacylase, deacylating Ala-tRNAPro at an ∼3.0-fold faster rate relative to Tb ProRS. MCP3 has a unique N-terminal domain (NTD) that we showed contributes to homodimerization but not to deacylation activity. Multiple-sequence alignments and an AlphaFold model of Tb MCP3 were used to identify putative catalytic residues. Alanine-scanning mutagenesis revealed Y97, R104, and K118 are essential for robust activity—we propose that these residues position the terminal A76 of tRNAPro into the editing site. Our data are consistent with the hypothesis that Tb evolved to encode functionally redundant and robust Ala-tRNAPro proofreading machinery to maintain translational fidelity at Pro codons during peaks in the intracellular alanine pool. MCP3 homologs are highly

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conserved in human pathogens and are not encoded in the human genome. Our structural and mechanistic insights into tRNAPro editing in Tb serves as the foundation for exploiting MCP3 as a drug target.

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Abstract 2278

Characterization of the dsRNA binding capabilities of Non-Structural Protein 1 of Influenza A from 1918 H1N1 Spanish Flu

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John Tomsho

Non-structural protein 1 of influenza A (NS1A) is a key virulence factor produced inside host cells infected with Influenza A Virus (IAV), and consists of an N-terminal dsRNA binding domain (RBD) and a C-terminal effector domain (ED), joined by a flexible linker. While NS1A is a highly promiscuous protein with a number of intracellular functions, it is primarily the non-specific dsRNA binding that enables influenza to evade our innate immune system; for this reason, NS1A has long been proposed as a potential drug target. Previous research in the field has demonstrated the necessity of dimer formation through the RBD to enable dsRNA binding, which is further enhanced by oligomerization through ED interactions. However, these studies were generally limited to either one truncated strain or used sequence-optimized dsRNAs. Here we utilize fluorescence polarization (FP) paired with fluorescence-based electrophoretic mobility shift assays (fEMSA) to characterize the dsRNA binding properties of the H1N1 strain of NS1A responsible for the 1918 Spanish Flu. We show that A/Brevig Mission/1/1918(H1N1) NS1A interacts with dsRNA through mechanisms not previously identified in other influenza strains. Through comparison with previously studied strains, mutational analyses are used to elucidate particular residues or regions responsible for these increased binding capabilities. These novel binding interactions may have contributed to the increased pathogenicity of the 1918 flu pandemic.

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Abstract 2286

Molecular characterization of the effect of RNA nucleoside modification on Flaviviral RNA dependent RNA polymerase *in vitro* transcription

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Daniel Eyler, Joshua Jones, Joyce Jose, Kristin Koutmou

RNA viruses utilize ribonucleic acid (RNA) as the archival genetic material. These viruses evolve mutations to enhance their biological fitness by balancing the integrity of their genome with their population level diversity. In addition to A, U, G, and C nucleotide substitutions, the cell's host proteins can create in situ biochemical modifications to nucleosides in the virus' RNA genome that have the potential to change viral mutation levels. Viral RNA-dependent RNA polymerases (RdRps) use hydrogen bonding base pair patterns to ensure faithful replication. RNA modifications can change nucleotide base pairing interactions between the template RNA and incoming nucleotides during viral transcription and replication, impacting the accuracy, processivity and speed of viral transcription and replication. We expect that the modifications that disrupt Watson-Crick base pairing between the template and incoming nucleotide will reduce the rate constants for NTP incorporation, while modifications which alter the equilibrium between base tautomers will decrease fidelity. To test this hypothesis, we investigated how naturally occurring nucleoside modifications alter the rate and fidelity of Flavivirus NS5 RNA-dependent RNA polymerases. To assess how nucleoside modifications impact the rate and fidelity of the Flavivirus NS5 RdRPs, we conducted *in vitro* RNA synthesis assays with over expressed and purified RdRps. NS5 clones were constructed for both the full length NS5 and RdRP polymerase domains. The *in vitro* RNA synthesis assays were carried out with purified NS5 and synthetic fluorescently labelled RNA templates with defined modified nucleosides designed to determine how RNA modification impacts viral RNA synthesis. Single nucleotide modifications were positioned in templates directly downstream of transcription initiation. The modified templates were designed based upon nucleoside modifications observed in Flavivirus RNA through UHPLC-MS/MS. This work will establish a set of "rules" that govern how individual nucleoside functional groups contribute to how the flaviviral RNA dependent RNA polymerase reads its RNA template, and provide molecular level insight into how modifications can contribute to viral evolution.

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Abstract 2294

RNA synthesis by a translesion DNA polymerase mediates RNA base damage repair

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Endogenous and environmental stressors such as reactive oxygen species (ROS), UV radiation, and chemical agents can damage DNA and RNA to compromise the integrity of the genome and transcriptome respectively, leading to cancer and neurodegenerative diseases. RNA and DNA damage can co-occur on an RNA-DNA hybrid in an R-loop generated during gene transcription that is actively involved in genome instability, cancer, and neurodegeneration. However, it is unknown if DNA and RNA damage can synergize to disrupt the integrity of DNA and RNA and if DNA and RNA damage repair can coordinate to remove the damage and resolve an R-loop. This study aims to elucidate how an RNA base damage repair pathway coordinates with DNA base excision repair (BER) to maintain RNA and DNA integrity during transcription stalling. We hypothesize that during gene transcription stalling, repair DNA polymerases perform RNA synthesis and coordinate with their DNA synthesis to accomplish RNA and DNA base damage repair. Using the strand break-mediated RNA modification (SBRM) profiling assay and *in vitro* biochemical assay, we initially detected different types of RNA base damage on the transcript of DNA polymerase b (pol b), a key BER enzyme. We then characterized the translesion DNA polymerase-mediated RNA synthesis during RNA base damage repair. We found that the oxidative stressor, potassium bromate induced oxidized RNA base damage such as 8-oxoguanine and abasic site sites predominantly located at Gs, As, and Cs in the encoding region of the pol b transcript. We showed that the translesion DNA polymerase h (pol η) possesses RNA synthesis activity to perform RNA synthesis during RNA base damage repair with high fidelity. We also found pol η RNA synthesis activity was significantly increased in the presence of manganese, a cofactor of DNA polymerases. Our results suggest that the RNA synthesis mediated by pol h can prevent transcription stalling and repair RNA base damage, leading to the resolution of R-loops, maintenance of the stability of DNA and RNA, and prevention of diseases. Our study will create a new paradigm to understand RNA damage repair pathway via DNA BER enzymes during transcription.

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Abstract 2298

Long noncoding RNAs play key roles in inflammation and metabolism in macrophages in human

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Long noncoding RNAs (LncRNAs) are a class of transcripts that are transcribed but remain mostly untranslated. Emerging evidence suggests that LncRNAs play crucial roles in regulation of gene expression, cell signaling, and metabolism. Recently, we demonstrated that lncRNA HOTAIR, which is well-known as a transcriptional repressor, plays key roles in regulation of cytokine expression and inflammatory response in macrophage. In a recent study, using RNA-seq analysis (in LPS-stimulated THP1-derived macrophage, THP1-MΦ and in primary BMDMs), we have discovered a series of novel lncRNAs (termed as Long-noncoding inflammation associated RNA, LinfRNA) that are closely linked to inflammatory responses in macrophages. Our study showed that, similar to cytokines and pro-inflammatory genes, human LinfRNA (hLinfRNAs) expression are induced (or downregulated) by LPS or other inflammatory mediators and live bacterial infections. hLinfRNA expression patterns vary with different types of stimuli suggesting their potential specificities toward different inflammatory signals and diseases. Antisense-mediated knockdown of hLinfRNA1 (ADORA2A-AS1) suppresses the LPS-induced cytokine expression, suggesting its critical roles of hLinfRNA1 in cytokine regulation and inflammation. Glucose metabolism and lipid metabolism play critical roles in macrophage activation and inflammation. We are investigating the role of hLinfRNAs in regulation of glucose metabolism in macrophage under inflammation. Indeed, our studies demonstrated that lncRNA HOTAIR play critical roles in Glut1 expression, glucose uptake and metabolism, Here we will present our recent finding on role of hLinfRNAs in regulation of inflammation, macrophage activation and glucose and lipid metabolism. Overall, we discovered a series of novel hLinfRNAs that are potential regulators of inflammation and metabolism in macrophages under inflammation and our studies may reveal novel lncRNA based biomarker and therapeutic targets for inflammatory and metabolic diseases.

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Abstract 2315

How Fitness Contributes to the Immune System of Breastfed Children

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Many women prioritize a healthy lifestyle while pregnant, but we don't often consider what this kind of lifestyle may contribute to a child after birth. Breastfeeding allows mothers to provide their children with important nutrients and hydration, it is also a means of communicating vital genetic information for the baby's developing immune system. Important immune factors have previously been found in breast milk, such as immunoglobulins, growth factors, cytokines, and several non-coding RNA species. Previous studies have demonstrated the presence of microRNAs, circular RNAs, long-coding RNAs, tRNAs, and rRNAs, but the levels of these had not yet been accurately measured. In this study, twenty women of differing fitness levels donated breast milk samples and underwent assessment of their overall fitness using BMI, relative peak oxygen consumption, body fat percentage, handgrip strength, visceral adipose tissue, lean mass index, and fat mass index. The breastmilk samples were sent off to a third-party lab to assess them and the data collected was analyzed by our lab. We confirmed the presence of snoRNAs, microRNAs, ncRNAs and tRNAs in breast milk, measured their levels, and compared the different types of RNAs and their levels in mothers of differing fitness levels. Our data shows a possible correlation of mothers with higher fitness levels producing higher percentages of RNAs in their breastmilk, but more data needs to be collected and analyzed to confirm.

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Abstract 2325

RNA Binding Characterization of the RRM and RRM-RGG2 Domains of EWSR1

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Emily Selig, Carmel Tovar, Matthew White, Renee Hoffman, Rachell Booth, David Libich

EWSR1, encodes an RNA-binding protein, EWS, which is composed of 615 amino acids and is divided into seven different domains with specific functions. A chromosomal rearrangement in which half of the EWSR1 gene is fused with one of several transcription factors of the E-twenty-six (ETS) family creates an aberrant oncogene called EWS-FLI1, which activates transcription of genes that cause tumorigenesis and interferes with the normal and essential functions of the remaining copy of EWS. This translocation is the driving factor for the development of Ewing sarcoma, a rare type of cancer that occurs in children and young adults. If left untreated, tumors can spread to other parts of the body. Understanding the normal functions of EWS will help in understanding how the EWS-FLI1 oncoprotein interferes with these processes to drive tumorigenesis and is of vital importance for developing treatments for Ewing sarcoma. The domains of EWS can be expressed and studied independently. In this study, the RNA/DNA recognition motif (RRM) fused with the arginine/glycine rich (RGG) domain of EWS was purified from *E. coli* and its interactions with various nucleic acid structures were investigated using gel shift assays and nuclear magnetic spectroscopy. Our preliminary results demonstrate an increase in binding affinity of RNA hairpin loops to the RRM-RGG2 domains over the RRM domain alone. Additional nucleic acids including G-quadplex and DNA:RNA hybrids structures are currently being analyzed.

This study was funded in part by the NIGMS R01GM140127 (DSL), GCCRI Startup Funds (DSL), and Welch Foundation BN-0032 (REB & RB). This work is supported by Structural Biology Core Facilities, a part of the Institutional Research Cores at UT Health SA funded by the Office of VPR and the Mays Cancer Center Drug Discovery & Structural Biology Shared Resource (NIH P30 CA05417X4).

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Abstract 2336

Biochemical and genetic dissection of the RNA-binding surface of the FinO domain of *Escherichia coli* ProQ

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RNA-binding proteins play important roles in bacterial gene regulation through interactions with small RNAs (sRNAs) and mRNA 5′ and 3′ UTRs. The ProQ protein is known to bind a wide pool of RNA molecules in *Escherichia coli*, but there are many open questions about the molecular mechanisms of ProQ-RNA interactions. Using a genetic bacterial three-hybrid (B3H) assay that reports on the interaction of ProQ with RNAs inside of *E. coli* cells, we previously found that the highly conserved "concave" face of the N-terminal FinO domain ProQ (ProQ-NTD) is the primary binding site for the RNA ligands SibB and *cspE*. Intriguingly, however, an NMR structural model for *E. coli* ProQ predicts that a single residue required for interaction with both RNAs, Arg80, is located on the opposite face (the "convex" face) of the NTD from the other residues involved in RNA binding. Based on this previous work, three important questions remained: 1) Is there indeed a role for the convex face of the ProQ-NTD in binding RNA? 2) Which of the residues that contribute to B3H interactions *in vivo* do so through direct RNA binding? 3) Will these RNA-binding residues contribute equally to interactions with a wide range of ProQ RNA ligands beyond the few RNAs studied so far? Answering these questions is critical to building a mechanistic model for ProQ-RNA interactions. In order to determine whether there is an important role for the convex face of the ProQ-NTD in RNA binding, we used our B3H assay to shed light on the *in vivo* structure of the ProQ-NTD. We used the highly conserved Arg80 residue as a foothold to conduct an unbiased forward genetic screen to identify compensatory mutants that reveal its *in vivo* location and were able to identify several compensatory mutants from saturation mutagenesis libraries that partially rescue RNA binding of an R80K variant. The results of this unbiased screen support a structural model in which, inside *E. coli* cells, the side chain of Arg80 resides on the concave face of the ProQ-NTD, along with other residues we previously identified as required for RNA interaction. This work demonstrates the power of a genetic assay to both generate hypotheses and test models regarding RNA-protein interactions using both forward and reverse genetics. To further explore the mechanism of RNA interaction by ProQ, we wanted to identify what residues important for *in vivo* RNA interactions act through direct RNA contacts. We utilized an *in vitro* gel-shift assay to analyze how mutations in positions of different evolutionary conservancy in the isolated ProQ-NTD affect its binding to RNA molecules *in vitro*. We compared the binding of ten ProQ-NTD variants to seven RNAs that are known to be ligands of ProQ in *E. coli*, and identified substitutions with

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strong, moderate and small negative effects on binding – some which affect all RNAs tested, and some uniquely contributing to the binding of certain RNAs. Overall, our work advances a model in which the concave face of the FinO domain serves as the main RNA binding site of *E. coli* ProQ and, for the first time, validates and extends our genetically-guided model with *in vitro* biochemistry.

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Abstract 2377

RbsD as a competing endogenous RNA and novel regulator of RpoS

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RpoS is a sigma-factor protein in *Escherichia coli* which accumulates during stressful conditions as cells enter stationary phase. It regulates a global stress response which can induce cell dormancy, promote antibiotic resistance, and initiate DNA repair. Several sRNAs (DsrA, RprA, and ArcZ) synthesized under different stress conditions play a critical role in stimulating rpoS translation. Depending on the stress condition, the sRNA binds the chaperone protein Hfq which opens a stem-loop in the rpoS transcript, exposing a ribosome binding site. A screen for novel regulators of RpoS was carried out with an RpoS'-'LacZ fusion and an overexpression library. The mRNA of the rbsD gene which encodes a ribose pyranase was found to reduce RpoS levels, although the RbsD protein was not necessary for the effect. Through beta-galactosidase assays, we determined that rbsD requires the rpoS hairpin loop for regulating RpoS translation. We also assessed different combinations of sRNA knockouts and found that in the absence of DsrA and ArcZ, the effect of rbsD overexpression was diminished. Silent mutations in the DsrA binding site partially disrupted RbsD control of RpoS. Moreover, it was found that only the 80 bp binding site region in rbsD is sufficient for the control of RpoS expression. In summary, RbsD mRNA has been found to be a novel regulator of RpoS through its interactions with DsrA and ArcZ, highlighting how mRNAs can serve multiple functions including the titration of sRNAs.

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Abstract 2441

Oligonucleotide-directed proximity-interactome mapping (O-MAP): A unified method for discovering RNA-interactions and probing RNA-scaffolded compartments within intact cells

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Within the context of the living cell, very little RNA is naked. RNA molecules form complex, dynamic networks of molecular interactions that underlie a host of biochemical functions, and which are central to organizing subcellular compartmentalization. In humans, for example, RNAs are key determinants of chromatin folding, and they nucleate and scaffold a host of biomolecular condensates that collectively control cellular metabolic, epigenetic, and stress-signaling pathways. But, characterizing these structures—identifying the biomolecules within an RNA's subcellular microenvironment—remains technically cumbersome. To address this challenge, we here introduce oligonucleotide-mediated proximity-interactome mapping (O-MAP), a straightforward and flexible method for identifying the proteins, RNAs, and genomic loci near a target RNA, within its native cellular context. O-MAP uses programmable oligonucleotide probes to deliver proximity-biotinylating enzymes to a target RNA. These enzymes then pervasively label all nearby (~20 nm) molecules, enabling their enrichment by streptavidin pulldown. O-MAP induces exceptionally precise RNA-targeted biotinylation, and its modular design enables straightforward validation of probe pools and real-space optimization of the biotinylation radius, thus overcoming key technical challenges for the field. Moreover, O-MAP can be readily ported across different target RNAs and specimen types, including patient-derived organoids and tissue samples. And, O-MAP achieves this without complex cell-line engineering, using only off-the-shelf parts and standard manipulations. Using a small cohort of model RNAs, we have developed a robust O-MAP toolkit for proteomic (O-MAP-MS), transcriptomic (O-MAP-Seq) and genome interaction (O-MAP-ChIP) discovery. O-MAP of the 47S-pre-rRNA—the long noncoding RNA that scaffolds the nucleolus—enabled a comprehensive "multi-omic" analysis of this subnuclear structure, and revealed hundreds of novel nucleolar protein-, RNA-, and chromatin interactions. O-MAP of XIST—the master regulator of X-chromosome inactivation—revealed novel RNAs that may play a role in this process, and unanticipated interactions between XIST and other chromatin-regulatory RNAs. Finally, we applied O-MAP to dozens of other target RNAs with varied abundance and localization—including mRNAs, lncRNAs, snRNAs, and introns within nascent transcripts—and observed highly precise RNA-targeted biotinylation in each case. These results were easily ported across cell lines and specimen types, including patient-derived organoids and cryo-preserved tissues. Given these results, we believe that O-MAP will be a powerful tool for elucidating the mechanisms by which RNA molecules drive subcellular compartmentalization in time and space, with particular impact on our understanding of nuclear architecture. Moreover, with O-MAP's precision, flexibility, and ease, we anticipate its broad use in studying countless other RNA phenomena throughout biology, and as a clinical diagnostic- and discovery tool.

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Abstract 2454

RbsD mRNA and ribose lower RpoS translation

Celeste Peterson, *Suffolk University*

Isabel Smith, Andrew Badaoui, Melisa Balla, Julia Lockart, Mikayla Cavanaugh

The *E. coli* sigma factor RpoS accumulates in stationary phase and activates transcription of the general stress response. To find novel regulators of RpoS, a screen with an RpoS'-'LacZ reporter was carried out with an overexpression library. Overexpression of the rbsD gene was found to reduce RpoS levels. However, a functional RbsD ribose pyranase protein was not necessary for the effect, indicating that the rsbD mRNA was carrying out the regulation. We used a series of LacZ fusions and RT-qPCR to determine how rbsD mRNA affects RpoS. We found that most of the regulation of RpoS occurred at the level of translation, with a modest effect on RpoS mRNA. Furthermore, the effect of rbsD overexpression was diminished in a reporter strain that did not have the RpoS untranslated hairpin loop or the small RNA chaperone Hfq. RbsD mRNA has previously been shown to bind to the sRNA DsrA, which also regulates RpoS. We found that the effect of rbsD on RpoS was dependent on DsrA and ArcZ and partially dependent on the DsrA binding sites on the rbsD mRNA. In addition, expression of just the DsrA binding sites on rsbD was sufficient for regulating RpoS. Finally, we found that sugar ribose, which induces RbsD levels, also lowers RpoS levels modestly, in a manner that requires RbsD. In summary, our data suggests that ribose lowers RpoS levels at post-transcriptional level through induction of rbsD mRNA which binds the sRNA DsrA and ArcZ. This work highlights the dual role of endogenous mRNAs in coding for proteins and also regulating signaling pathways.

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Abstract 2460

Development of a highly-sensitive RT-qPCR assay for the detection and quantitation of siRNAs to further understand their role in mRNA quality control in *Caenorhabditis elegans*

Juliane Castellani, *Villanova University*

Elaine Youngman

Mutations to the genetic material or errors in RNA processing can result in production of low-quality mRNA transcripts that, for example, contain premature stop codons or lack a stop codon altogether. Cells across all kingdoms of life have therefore evolved mRNA surveillance, or quality control, mechanisms that trigger mRNA decay to mitigate potential toxicity resulting from translation of these low-quality mRNAs. Full understanding of the mechanisms underlying these quality control pathways is still developing, and previous work in our lab suggests there is a role for small noncoding RNAs in the nonstop mRNA decay pathway in the nematode *C. elegans*. Briefly, we have shown that loss of a stop codon at the gene F43E2.6 is necessary but not sufficient to trigger the production of endogenous siRNAs (22G RNAs) that target the F43 mRNA in several wild isolates of *C. elegans*. Genetic data indicate that in the reference strain (N2), a locus on Chromosome II represses nonstop-dependent production of F43-targeting 22G RNAs. To uncover the identity of this gene, I am developing a robust RT-qPCR assay for the detection and quantitation of 22G RNAs and other small noncoding RNAs. Previously, siRNAs have been difficult to amplify through PCR due to their small size, being about the same size as a traditional PCR primer. I will present development and quantitative comparison of two methods, based on reverse transcription with a stem loop primer or on polyadenylation of small RNAs, and will discuss progress in identification of candidates for the nonstop-siRNA repressor gene.

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Abstract 2461

Developing a robust system for genetic detection of RNA-binding protein

Suxuan Wang, *Mount Holyoke College*

Chandra Gravel, Katherine Berry

RNA-binding proteins play important roles in gene regulation across all domains of life. In order to detect and characterize bacterial RNA-protein interactions in a native cellular environment, our laboratory has developed a bacterial three-hybrid (B3H) assay that connects the strength of RNA-protein interactions inside of *Escherichia coli* cells to the activity of a reporter gene. In this B3H assay, plasmids encoding two hybrid proteins (DNA-RNA "adapter" and RNAP-bound "prey") and one hybrid RNA ("bait") are transformed into *E. coli* reporter cells and the strength of the RNA-protein interaction is linked to the transcriptional output of a reporter gene. With this system, interacting RNAs and proteins do not need to be purified and their interactions can be assessed inside living bacterial cells. Since its inception, our B3H assay has been used exclusively with a lacZ reporter, encoding β-galactosidase. While β-gal assays and blue/white screens are effective for testing the effects of site-directed mutants, screening small libraries, several exciting potential assay applications would require alternate reporter genes and detection methods. In order to expand the applications of the B3H assay to larger library sizes, this study aims to develop new reporter systems for the transcriptional reporter used in the assay. We have designed and cloned dual-fluorescent reporters to quantitatively report on the real-time protein-RNA interactions *in vivo* and provide an intrinsic negative control. These initial constructs show promising results in the detection of protein-protein interactions using a related two-hybrid assay. Ongoing work aims to optimize the genetic background of these reporters and benchmark them using a variety of fluorescence detection methods against established B3H interactions between ProQ and Hfq and their respective RNA ligands. The ability to utilize fluorescence-activated cell sorting (FACS) to examine larger library sizes will be an important advance in expanding the applications of our B3H assay, and support our ongoing goals of dissecting the mechanisms of RNA-binding proteins involved in gene regulation.

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Abstract 2497

New tools for detecting mRNA methylation

Kate Meyer, *Duke University School of Medicine*

m6A is the most abundant internal mRNA modification and plays important roles in regulating RNA function. However, methods for detecting m6A in cells have suffered from the need for large amounts of input RNA. Recently, our lab developed DART-seq (deamination adjacent to RNA modification targets) to overcome this challenge. DART-seq uses a fusion protein consisting of the m6A-binding YTH domain tethered to the cytidine deaminase APOBEC1. When this APOBEC1-YTH fusion protein encounters methylated RNA, it binds to m6A and directs C-to-U editing at nearby cytidine residues. We have demonstrated the ability of DART-seq to identify m6A sites transcriptome-wide using low amounts of RNA *in vitro*. Moreover, we have shown that expression of APOBEC1-YTH in cells can achieve single-cell m6A identification, revealing new insights into m6A biology that have been missed by other m6A profiling methods. Finally, application of DART-Seq to the mouse brain has revealed previously unknown features of m6A biology in distinct cell types.

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Abstract 2513

RNA modifications at the virus-host interface

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RNA-based regulation of viral RNA genomes occurs throughout infection. We have previously found that the RNA modification N6-methyladenosine (m6A) regulates infection by positive-strand RNA viruses in the Flaviviridae family, including hepatitis C virus (HCV). This regulation occurs by both m6A and the m6A-machinery proteins, which can act on either cellular or viral RNA to regulate infection. For HCV, m6A on viral RNA to controls the viral lifecycle. However, it has remained unknown how m6A is added to HCV RNA as the HCV lifecycle occurs solely in the cytoplasm, while m6A addition on cellular mRNA occurs in the nucleus. Recently, we found that addition of m6A to HCV RNA by the m6A-methyltransferase proteins METTL3 and METTL14 requires the cellular protein WTAP. WTAP is part of cellular mRNA m6A-methyltransferase complex and targets METTL3 to cellular mRNA. Interestingly, we found that HCV enriches WTAP localization in the cytoplasm. Further, WTAP controls both METTL3 interaction with HCV RNA and m6A modification of the HCV RNA genome. Excitingly, WTAP regulation of both HCV RNA m6A modification and virion production are independent of its ability to localize to the nucleus, revealing that m6A regulation of HCV RNA occurs in the cytoplasm. Our ongoing studies are focused on defining new methods to profile m6A on HCV RNA and identifying new regulators of m6A on HCV RNA. Ultimately, a detailed understanding of the interactions that act at the m6A virus-host interface will uncover novel strategies to develop antiviral therapies to target this RNA regulatory control that is exploited by RNA viruses for their replication.

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Abstract 2556

Downregulation of miR-30a-5p promotes progression of Non-Small Cell Lung Cancer by targeting MTDH gene

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Erick Thokerunga, Christian Cedric Bongolo, Jiancheng Tu

Background: Dysregulated microRNA expressions promote Non-small cell lung cancer (NSCLC). Understanding the mechanisms by which they achieve this could be vital for drug discovery.

Objective: This study aimed at determining the roles and mechanism of action of miR-30a-5p in NSCLC.

Methods: Real time-quantitative polymerase chain reaction was conducted to determine miR-30a-5p expression in NSCLC cell lines. Cell proliferation, migration and invasion were determined using Cell Counting Kit-8, Transwell, and wound healing assays respectively. Bioinformatics predicted miR-30a-5p gene target which was confirmed with RT-qPCR, western blot and luciferase reporter assay. MiR-30a-5p cancer activity was further assessed *in vivo*.

Results: MiR-30a-5p was downregulated in NSCLC cell lines. Restoration of expression suppressed NSCLC cell proliferation, migration and invasion *in vitro*, and tumor growth *in vivo*. Metadherin (MTDH) was found to be its direct target gene in NSCLC cells. Its 3′UTR region possesses complementary sequence to miR-30a-5p confirmed by luciferase reporter assay. Its expression in NSCLC was inversely correlated with miR-30a-5p expression. Lastly, its upregulation *in vivo* by inhibiting miR-30a-5p promoted tumor growth and vice versa.

Conclusion: These findings all but suggest that miR-30a-5p regulates NSCLC progression by targeting MTDH through the miR-30a-5p/MTDH axis. This axis could be a drugable target in NSCLC.

Keywords: Downregulation; MTDH, miR-30a-5p, lung cancer, progression.

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Abstract 2559

Hyperactivation of an RNA Binding Protein by Cancer-associated Mutations

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RNA binding proteins (RBPs) control proper gene expression by directly and specifically binding RNA to modulate the identity and fate of transcripts. Abnormal transcriptomes are a hallmark of cancer and several RBPs have been identified in tumors to contain hotspot mutations. We evaluated the consequences of cancer-associated hot-spot mutations in poly C binding protein 1 (PCBP1), a ubiquitous and highly expressed RBP, on RNA processing. PCBP1 hotspot mutations are primarily found in colorectal adenocarcinoma. Using a variety of biochemical, structural, and cell-based approaches we found that hotspot mutations in PCBP1 led to a hyperactivated state that enhanced RNA binding and regulation. We demonstrated that the hyperactivated state was driven through tertiary structural changes which led to improper mutant PCBP1 oligomer formation. Transcriptome-wide, we found that mutant PCBP1 bound specific mRNAs in pathways known to be involved in colon cancer. Overall, our work establishes the molecular mechanisms and regulatory consequences of PCBP1 hyperactivation in colon cancer and is likely to have implications for other mutant RBPs.

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Abstract 2580

Regulation and Functions of Circular RNAs

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Circular RNAs (circRNAs) are single-stranded RNAs that lack ends. More than 100,000 circRNAs have been identified from human cells, but most of them have unknown functions. CircRNAs also lack many of the regulation found on linear RNAs, such as 5′ cap and 3′ polyA-tail. How cells regulate circRNAs and what are their biological roles are open questions in the field. We are building and applying technologies to isolate, identify, and study endogenous and engineered circRNAs. We have previously found that the RNA modification N6-methyladenosine (m6A) is a molecular marker for "self" circRNAs to distinguish from foreign, potentially pathogenic, circRNAs. Unmodified circRNA, but not m6A-modified circRNA, activates innate immune receptor RIG-I to trigger an immune signaling cascade. We also demonstrated that engineered circRNAs are potent adjuvants to induce antigen-specific T cell activation, antibody production, and anti-tumor immunity *in vivo*, and m6A modification suppresses immune gene activation and adjuvant activity. We are investigating additional features on circRNAs that enables cells to differentiate self and non-self circRNAs. In addition, we are also identifying novel circRNAs that are encoded by viruses or hosts, and elucidating their regulation and functions.

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Abstract 2592

Trypanasoma cruzi dystegulates piRNA computationally predicted to target proinflammatory molecules in Primary Human Cardiac Myocytes during Early Infection Phase

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Kayla Rayford, Ashutosh Arun, Pius Nde, Siddharth Pratap

Trypanosoma cruzi, the etiological agent of Chagas disease, is an intracellular protozoan parasite, which is now present in most industrialized countries. About 40% of *T. cruzi* infected individuals will develop severe, incurable cardiovascular, gastrointestinal, or neurological disorders. *Trypanosoma cruzi* dysregulates the gene expression profile of primary human cardiomyocytes (PHCM) during the early phase of infection through a mechanism which remains to be elucidated. The role of small non-coding RNAs (sncRNA), including PIWI-interacting RNA (piRNA), play in regulating gene expression during the early phase of infection is unknown. To understand how *T. cruzi* dysregulate gene expression in the heart, we challenged PHCM with *T. cruzi* trypomastigotes and analyzed piRNA expression with RNA-Seq. The parasite induced significant differential expression of host piRNAs, which we computationally predicted to target and regulate the genes which are important during the early infection phase. The parasite induced differential expression of 217 unique piRNAs. Of these differentially expressed piRNAs, 6 were known and 211 were novel piRNAs. Our analysis showed that some of the dysregulated known and novel piRNAs could target and potentially regulate the expression of pro-inflammatory genes including NFATC2, FOS and TGF-β1, reported to play important roles during *T. cruzi* infection. Further evaluation of the specific functions of these piRNAs in the regulation of gene expression during the early phase of infection will enhance our understanding of the molecular mechanism of *T. cruzi* pathogenesis. Characterization of the regulatory interactions between piRNAs and pro-inflammatory target transcripts and can be exploited for future biomarker discovery and development of therapeutics.

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Abstract 2619

Translocation Mechanism of the DEAH-box Helicase Prp22

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The DEAH-box helicase Prp22 is an ATP-dependent helicase essential for the splicing of precursor messenger RNA (pre-mRNA) into mature mRNA by the spliceosome. ATP-hydrolysis by Prp22 is required for release of mature mRNA from the spliceosome after intron cleavage. In addition, Prp22 helps maintain splicing fidelity by proofreading intronic consensus sequences and discriminating between suboptimal 3′ splice sites during the 2nd step of splicing catalysis. While previous studies have revealed key aspects of the enzymatic activities of Prp22, such as NTP usage, substrate specificity, and polarity, its translocation and unwinding mechanisms remain elusive. Here we report a single molecule Förster resonance energy transfer (smFRET) study to characterize these Prp22 activities *in vitro*. Using smFRET labels on the RNA substrate, we probe substrate binding and unwinding in the absence and presence of ATP. Analysis of the resulting smFRET trajectories reveals the conditions that generate sufficient unwinding force to melt proximal RNA structures. Together, our results reveal mechanistic insights into how this DEAH- box helicase may apply force to the RNA at a distance to enable spliceosomal rearrangements necessary during pre-mRNA splicing.

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Abstract 2637

Thioester Electrophiles as a New Class of SHAPE Probes

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RNA is a multifunctional molecule capable of regulating gene expression, in large part because it can form a variety of RNA secondary and tertiary structures. The emergence of RNA viruses like SARS-CoV-2 emphasizes the need to accelerate our understanding of how viral RNA structure dictates its function. One approach to map RNA secondary structure, called Selective 2′-OH Acylation Analyzed by Primer Extension (SHAPE), utilizes select electrophiles that unbiasedly modifies the 2′-hydroxyl of riboses in unpaired nucleotides, forming adducts that are detected through a variety of sequencing methods. While SHAPE is widely utilized, most existing SHAPE reagents suffer from several drawbacks: 1) poor water solubility; 2) limited commercial availability; and 3) they function optimally when freshly synthesized, requiring synthetic organic expertise. To overcome these obstacles, our goal is to develop a user-friendly SHAPE reagent kit that provides highly reactive, soluble SHAPE reagents capable of probing RNA structure *in vitro* as well as *in vivo*. We present our investigations on developing thioester electrophiles as a new class of SHAPE reagents. Our reagent is prepared by mixing two stable components to generate the reactive thioester electrophile in situ. We report our preliminary results in model systems and the scope to expand the library of our reagents.

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Abstract 2656

Multi-functional aspects of RNA binding proteins in health and disease

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Gary Bassell

RNA transport and local translation provide spatial control of gene expression, and RNA binding proteins (RBPs) act as critical adapters in this multi-step process. Muscleblind-like (MBNL) RNA binding proteins, implicated in myotonic dystrophy and cancer, localize RNAs to myoblast membranes and distal neurites through unknown mechanisms. We found that MBNL forms motile and anchored granules in neurons and myoblasts, and selectively associates with kinesins Kif1bα and Kif1c through its zinc finger (ZnF) domains. Other RBPs with similar ZnFs also associate with these kinesins, implicating a motor-RBP specificity code. Live cell imaging and fractionation revealed that membrane anchoring is mediated through the unstructured carboxy-terminal tail of MBNL1. Both kinesin- and membrane-recruitment functions were reconstituted using MBNL-MS2 coat protein fusions. This approach, termed RBP Module Recruitment and Imaging (RBP-MRI), decouples RNA binding, kinesin recruitment, and membrane anchoring functions, while also establishing general strategies for studying multi-functional, modular domains of RBPs.

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Abstract 2659

5-methylcytosine (m5C) RNA modification controls the innate immune response to virus infection by regulating type I interferons

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5-methylcytosine innate immune response interferon virus infection, 5-methylcytosine (m5C) is one of the most prevalent modifications of RNA, playing important roles in RNA metabolism, nuclear export, and translation. However, the potential role of RNA m5C methylation in innate immunity remains elusive. Here, we show that depletion of NSUN2, an m5C methyltransferase, significantly inhibits the replication and gene expression of a wide range of RNA and DNA viruses. Notably, we found that this antiviral effect is largely driven by an enhanced type I interferon (IFN) response. The antiviral signaling pathway is dependent on the cytosolic RNA sensor RIG-I but not MDA5. Transcriptome-wide mapping of m5C following NSUN2 depletion in human A549 cells revealed a marked reduction in the m5C methylation of several abundant noncoding RNAs (ncRNAs). However, m5C methylation of viral RNA was not noticeably altered by NSUN2 depletion. In NSUN2-depleted cells, the host RNA polymerase (Pol) III transcribed ncRNAs, in particular RPPH1 and 7SL RNAs, were substantially up-regulated, leading to an increase of unshielded 7SL RNA in cytoplasm, which served as a direct ligand for the RIG-I–mediated IFN response. In NSUN2-depleted cells, inhibition of Pol III transcription or silencing of RPPH1 and 7SL RNA dampened IFN signaling, partially rescuing viral replication and gene expression. Finally, depletion of NSUN2 in an *ex vivo* human lung model and a mouse model inhibits viral replication and reduces pathogenesis, which is accompanied by enhanced type I IFN responses. Collectively, our data demonstrate that RNA m5C methylation controls antiviral innate immunity through modulating the m5C methylome of ncRNAs and their expression.

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Abstract 2692

Subnuclear localization of HCMV 5 kb RNA using Fluorogenic RNA Aptamer Broccoli

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Caroline Kulesza

Human Cytomegalovirus (HCMV) is a wide-spread pathogen currently infecting anywhere from 56% to 94% of the population depending on the country. It is characterized by its lifelong infection and ability to reactivate even after several years when its host is under stress. While typically harmless, it has a high mortality rate in immunocompromised individuals and when transmitted vertically from mother to fetus. Its ability to infect a wide variety of cells and establish latency in bone marrow cells, make it difficult to target with drugs. By acquiring more knowledge behind the mechanisms of switching latency off and on, it would be possible to prevent lytic replication from turning on and prevent symptomatic infection. 5 kb RNA is a gene of interest in HCMV because previous research has demonstrated that 5 kb RNA most likely plays a critical role in persistent infection of cells. 5 kb RNA homologs have been seen among every other type of betaherpesvirus, indicating its significance to that type of virus. Although it was determined that 5 kb RNA doesn't significantly affect lytic replication, knocking out its expression causes a remarkably reduced ability of the virus to persist in salivary cells. In a previous paper it was seen that recombinant viruses without functional 5 kb RNA could not persist in salivary glands within mice. This means that 5 kb RNA seems to significantly influence the virus's ability to remain in the host's body. However, its mechanism remains unclear. In order to characterize 5 kb RNA further, 5 kb RNA will be tagged with a fluorogenic RNA aptamer called Broccoli through allelic exchange. Human fibroblast cells will be infected with the recombinant HCMV and observed. In addition, the fluorogenic RNA, Broccoli, will be inserted in different locations within 5 kb RNA which may interfere with its function depending on the region, and may illuminate which regions of 5 kb RNA are most important to its function. Understanding the mechanism of 5 kb RNA may provide a new drug target for HCMV. Through these methods, we hope to localize and characterize 5 kb RNA further.

This project was funded by NIH Undergraduate Research Training Initiative for Student Enhancement (U-RISE) at Fort Lewis College.

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Abstract 2733

Mapping oxidative stress response RNA-protein networks

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In recent years, a wide variety of chemical modifications to RNA have been discovered. Moreover, the profile and abundance of RNA modifications has been found to be influenced by environmental stressors. Given its relevance to pathogenesis and to the onset of several diseases, understanding how oxidative stress remodels basic cellular pathways and contributes to new cellular phenotypes is a broad scientific goal. As supporting preliminary evidence, we have found that cellular RNAs can undergo significant 8-OG modifications when exposed to differential levels of various environmental mixtures; 8-oxoG is the most prevalent RNA modification associated with cellular exposure to oxidative stress. A fundamental question in understanding oxidative stress response networks is how RNA-binding proteins contribute to these transcriptional responses, particularly as it relates to the processing of oxidized RNAs in cells. In this talk, we will discuss our efforts on identifying RNA-binding proteins that have functional impact in the processing of oxidized RNAs and mechanisms by which they might selectively recognize these modified RNAs and exert their functions.

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Abstract 2742

Methyltransferase METTL8 is required for 3-methylcytosine modification in human mitochondrial tRNAs

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The 3-methylcytosine (m3C) modification is found on several different eukaryotic tRNAs within the anticodon loop. Interestingly, in mammals, there has been an expansion of m3C containing tRNAs to include mitochondrial (mt)-tRNA-Ser-UGA and mt-tRNA-Thr-UGU. Previous work has identified the Trm140 homologs responsible for m3C formation in human cytoplasmic tRNAs, however, the enzyme responsible for the catalysis of m3C on mitochondrial tRNAs has remained elusive. In this work, we demonstrate that the Methyltransferase-Like 8 (METTL8) protein is responsible for m3C formation in mt-tRNAs, which is dependent upon proper localization to mitochondria. We show human cells lacking METTL8 are deficient in m3C formation in mt-tRNAs, but not in the nuclear-encoded tRNAs. This deficiency in m3C formation could be rescued upon re-expression of full-length METTL8, but not upon expression of a METTL8 variant lacking the N-terminal mitochondrial targeting sequence. Additionally, we find a novel interaction between METTL8 and the mitochondrial seryl-tRNA synthetase. Notably, we also observe disruptions in the native migration pattern of mt-tRNA-Ser-UGA in METTL8-deficient cells compared to wild-type cells, suggesting a novel role for m3C in tRNA folding. This work identified METTL8 as a mitochondrial-associated protein that interacts and catalyzes m3C formation on mt-tRNA-Ser-UGA and mt-tRNA-Thr-UGU, while also highlighting a potential role of m3C in tRNA structure.

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Abstract 2749

Alternative structures of RNA control gene expression and offer therapeutic strategies

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RNA viruses are diverse and abundant pathogens responsible for numerous human ailments, from common colds to AIDS, SARS, Ebola, and other dangerous diseases. RNA viruses possess relatively compact genomes and have therefore evolved multiple mechanisms to maximize their coding capacities, often using overlapping reading frames. In this way, one RNA sequence can encode multiple proteins via mechanisms including alternative splicing and ribosomal frameshifting. Many such processes in gene expression involve the RNA folding into three-dimensional structures that can recruit ribosomes without initiation factors, hijack host proteins, cause ribosomes to frameshift, and expose or occlude regulatory protein binding motifs to ultimately control each key process in the viral life cycle. I will discuss the RNA structure of HIV-1 and SARS-CoV-2 and the importance of alternative conformations assumed by the same RNA sequence in controlling gene expression of viruses and bacteria.

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Topic Category Science Outreach Activity

Abstract 2502

B2AR: The Smooth Operator

Chris Chou, *Longmont High School*

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Adrenergic receptors function all throughout the body. In many cases, they send signals that put the body into "fight or flight" mode with implications for anxiety, asthma and heart problems. Understanding these receptors will allow scientists to create medicines that target adrenergic receptors in different body tissues. Because the human body has to respond to a wide variety of stimuli there are nine different receptors displaying different locations and functions. The beta-2 adrenergic receptor (β_2AR) is found in smooth muscle, airways and other tissues including muscular arterioles, iris constrictor muscles, uterus, veins, bronchi, liver, and pancreas. During the fight or flight response, epinephrine (adrenaline) binding to β2AR causes blood flow to the digestive system to be restricted, while heart rate and oxygen intake are increased. Epinephrine binding to the extracellular side of β2AR triggers a conformational switch to the β2AR active state, which allows its G-protein to bind to a positively charged binding site on the intracellular surface of β2AR. The β2AR receptor is thought to exist as a dimer in the plasma membrane. Within each monomer consisting of 403 amino acids, seven alpha helices wind through the cell membrane. Epinephrine binds the ligand-binding pocket from the extracellular side of β2AR which contains 14 amino acids: Trp109, Asp113, Phe193, Tyr199, Ser203, Ser204, Ser207, Trp286, Phe289, Phe290, Asn293, Tyr308, Asn312, and Tyr316. Transmembrane helices III, V, VI, and VII each have a proline-induced kink thought to enable membrane-spanning structural rearrangements required for activation of cytoplasmic G-protein effectors. β2AR also has a small, extracellular helix that contains two disulfide bonds between residues Cys106-Cys191 and Cys184-Cys190 that is not found in other G-protein coupled receptors. Further study of B2AR could yield higher efficacy prescription medications that mediate the effects of epinephrine in the body for treatment of disorders such as anxiety and depression. The Longmont High School SMART (Students Modeling A Research Topic) Team has designed a model of beta-2 adrenergic receptor using 3D printing technology to investigate structure-function relationships.

The Longmont High School SMART (Students Modeling A Research Topic) Team is funded and supported by the St. Vrain Valley School District (Longmont, Colorado).

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Abstract 2503

Keep Calm and Prion: The End is Near

Chris Chou, *Longmont High School*

Gayle Geschwind, Richard Martyr, Willa Conlin, Anna Kragerud, Annabelle Poole, Emma Schaub, Erik Swanson, Lola Trojanovich

Neurological diseases with unique and mysterious origins, good proteins gone bad to the bone: Mad cow disease, fatal familial insomnia, and other prion-caused neurological diseases are rare but almost always deadly. The human prion protein, distributed throughout human tissues, can assume two different conformations: the native form known as PrPC, and a misfolded form PrPSc. PrPC is primarily located in the central nervous system, anchored to the surface of nerve cells via glycophosphatidylinositol, a phosphoglyceride. The function of PrPC is not well understood, however it seems to be involved in several important roles including: protecting neurons from excitotoxicity (when neurotransmitters levels are high enough to cause cell death), maintaining a balance of copper (II) and zinc ions, as well as regulating neuronal signaling and circadian rhythm. PrPC interacts with a number of membrane receptors including ionotropic and metabotropic glutamate receptors, ion channels, and amino acid transporters. The conformational transition of PrPC to PrPSc can result in neurological diseases including: fatal familial insomnia, Creutzfeldt-Jakob disease, and Gerstmann-Sträussler-Scheinker syndrome. These infectious diseases are spread when PrPSc comes in contact with PrPC, causing PrPC to refold into PrPSc. There are three ways people contract these diseases: sporadic event (no identified cause), contact event (acquired by contact, such as consumption of infected meat), and genetic event (caused by inherited or acquired point mutation(s) in the prion gene). Symptoms of these diseases include irritation, hallucinations, muscle stiffness, and changes in behavior. PrPC possesses 209 amino acid residues after post-translational modification, during which 22 N-terminal amino acids are removed. PrPC contains a flexible N-terminal tail followed by 2 β-strands and 3 α-helices. PrPSc contains more β-strands than PrPC (α-helix rich) and aggregates into long amyloid fibrils. The different secondary structure of PrPSc results in different properties, such as resistance to proteolytic degradation and insolubility. Human PrPC differs slightly from other mammalian prion proteins, but cross species infection can occur. There are many opportunities for future research into PrPC and PrPSc that could lead to a better understanding of this protein and its related diseases. The structure of PrPC is not well understood and studies are difficult to conduct because it is insoluble and difficult to crystallize. Research into the structure could help explain why misfolded PrPC can cause different diseases. Evidence suggests that neurodegenerative diseases, such as Alzheimer's, may be entirely or partially caused by mutations in human prion proteins. Prion diseases also occur spontaneously, and the mechanism is unknown. Due to the high fatality of prion-related diseases and late intervention in most cases, it is difficult to study the diseases and conduct clinical trials to treat them. Earlier detection methods and improved therapeutics could enable researchers and physicians to study and treat these diseases and to elucidate the role of PrPSc. The Longmont High School SMART (Students Modeling A Research Topic) Team has designed a model of human prion protein using 3D printing technology to investigate structure-function relationships.

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ASBMB

Abstract 2510

Guardian of the Genome: p53 Tumor Suppressor

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Approximately 1.9 million Americans are diagnosed with cancer annually, and an estimated 50% of all cancers are directly affected by mutations in the p53 tumor suppressor protein. Otherwise known as the "guardian of the genome" p53 resides in the nucleus and is central to the regulation of cell division and cell death. Cancer-causing mutants of p53 fail to suppress the division of cancerous cells, which is why it is crucial to understand how these mutants occur and their effects on signaling pathways. The p53 protein binds specific sequences in genomic DNA, and it binds as a tetramer. Upon binding DNA, p53 can help activate specific sets of genes in response to stress or exposure, for example, DNA damage from X-rays. If a cell is unable to repair DNA damage, the p53 response may ultimately trigger apoptosis (programmed cell death) to prevent propagation of cells with damaged genomic DNA. Cancer-causing p53 mutants not only lose regular function, but can also gain oncogenic functions that facilitate tumor growth. The p53 homotetramer is formed as a dimer of dimers in which each individual monomer contains 393 amino acid residues. Three key domains of p53 have been studied in depth: the tetramerization domain, the DNA binding domain, and the transactivation domain. The remaining regions of p53 are intrinsically disordered, flexible, and unstructured. Consequently, no structural data is available for them. Cancer-causing mutations are most commonly found in the DNA binding domain, with key sites including arginine residues 175, 249, 273, and 282. When these positively charged residues are mutated, they no longer bind to the negatively charged DNA backbone, thereby disabling the protective regulatory functions of p53. The p53 protein is an extremely promising target for cancer research, and studies have shown that it is possible to rescue wild type p53 function from specific mutants. One of the most promising drug targets is the p53 Y220C mutant, which is considered an excellent pharmaceutical target in personalized medicine, with a potential drug currently in Phase I clinical trials. The Longmont High School SMART (Students Modeling A Research Topic) Team has designed a model of p53 using 3D printing technology to investigate structure-function relationships.

The Longmont High School SMART Team is supported and funded by the St. Vrain Valley School District.

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Abstract 2546

Precision Medicine, Ancestry, Genetics and Race

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Over the past two decades, two seemingly contradictory trends have emerged in biomedical research. On the one hand, it is now generally recognized that "race" is a social construct rather than a biological variable. On the other, there is increasing evidence that epidemiology and precision medicine research require diverse cohorts that adequately represent ethnic/racial groups that have been traditionally understudied in biomedical research. Genomics can help us resolve this apparent contradiction. Human complex traits, including diseases and response to medications, result from the interaction of genes and environment. "Environment" includes socioeconomic disparities, lifestyles, diet, chemical, physical and microbiological exposures as well as the physiological effects of behavioral exposures such as chronic stress. These factors act upon diverse human genomes, which originally evolved in response to local selective pressures that may or may not still operate in different regions in contemporary society. Genetic variants that were adaptive in their original environments may be maladaptive in a different environment. Epigenetic modifications and transcriptional or post-transcriptional responses to external stimuli (e.g., diet, chemical pollutants) mediate the interaction between genes and environment. Recent large-scale studies support the notion that most human diseases have a heritability component. However, with the exception of well-recognized monogenic diseases, this heritability is generally polygenic and individually variable. This contributes to explain why attributing genetic significance to social labels such as race or ethnicity is a scientific fallacy. First, genomes of African ancestry show the largest individual variability among ancestry groups, consistent with the fact that humans originally evolved in Africa. Hence, there is no such thing as a genetically homogeneous "African race". Second, the vast majority of African-Americans are genetically admixed with variable fractions of European genes, and it's impossible to determine the local ancestry of a given individual at each locus without detailed genomic analysis. Third, "Hispanics" have continuously variable ancestry fractions including Native American, European and African genes in virtually every possible ratios, which vary among individuals and regions of origin in Latin America. Fourth, the term "Asian/Pacific Islander" includes multiple, ancestrally distinct populations and it has been used with different meanings in different countries. In summary, the only way to accurately estimate genetic risk factors for complex traits in a given individual is to analyze his/her individual genome. This is why large cohorts including individuals of diverse ancestries are necessary to accurately identify genetic determinants of health outcomes. The future of precision medicine and precision prevention rests on detailed genomic and environmental analysis of diverse individuals within large, inclusive cohorts, rather than on imprecise labels influenced by social and political factors.

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Abstract 2557

Restorative Inclusive Excellence: An organizational approach to a sustainable equitable and inclusive scientific enterprise

Amanda Bryant-Friedrich, *Wayne State University*

Through the combination of practices rooted in restorative justice with a systemic and individual exploration and adaptation of equity-mindedness a sustainable culture of inclusive excellence will be achieved. Inclusive excellence is defined as the act of establishing hallmarks of excellence and organizational effectiveness; operationalizing inclusion across organizational functions; and creating education and professional development processes that have diversity equity and inclusion at their core. Many organizations wish to achieve this goal, but find it difficult to find a sustainable path for cultural integration. The medical and biomedical communities have struggled for decades despite much discussion, research and investment. This presentation will explore the use of restorative practices, equity-mindedness and a well establish theory of organization management, inclusive excellence, to bring these communities closer to this goal. Restorative Inclusive Excellence (RIE) represents an organizational approach which centers the prevention of and response to harm inflicted on individuals and/or a community by practices rooted in racism, bias, an absence of equity and intentional or unintentional exclusion. To fully prepare a community for RIE, it is necessary to illuminate the level of equity-mindedness at the individual, micro and macro levels of an organization. Equity-minded requires a perspective, at all levels, that facilitates the recognition of inequity in outcomes. Restorative practices provide a framework for the identification, prevention and response to harm by helping individuals build relationships that strengthen their communities. Key to the successful implementation of these practices is the establishment of inclusive and collaborative communication and decision making. Through the use of restorative practices, policies, practices and procedures which allow the perpetuation of systemic racism, inequity and exclusion can be restored to provide equity and inclusion in diverse communities.

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Abstract 2571

Animal Model of Contusion Compression Spinal Cord Injury by Yasargil Aneurysm Clip

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Dwikora Utomo, Heri Suroto

Introduction: Animal spinal cord injury (SCI) models have shown to be invaluable in better understanding the mechanisms related to traumatic SCI and evaluating the effectiveness of experimental therapeutic interventions. The use of clip compression can produce contusion–compression SCI models in rats with clinical features in the form of total paralysis, retention of micturition, and retention of defecation. This study aimed to validate the effects of the duration of Yasargil aneurysm clip application on the formation of SCI models with analyzed neuropathic pain, locomotor function, histology, and tumour necrosis factor (TNF)-α.

Methods: True experimental study investigated 20 Sprague Dawleys divided into normal, 30-second, 60-second, and 90-second groups. Contusion–compression model of SCI post-laminectomy was done in 0, 30, 60, and 90 seconds using an Yasargil aneurysm clip, with a force of 65 g (150kDyne). The data was recorded by blinded evaluators. The study's protocol was reviewed and approved by the university (REC.1112/UN25.8/KEPK/DL/2021). All rats were approved by the Animal Health Office (no., 503/ A.1/0005.B/35.09.325/2020).

Result: We found that the locomotor expression did not indicate total paralysis after compression durations of 0 and 30 seconds, while the compression durations of 60 and 90 seconds could result in total paralysis. There was no significant difference in the mean BBB scores between the compression durations of 60 and 90 seconds with $p = 1.000$ ($p > 0.05$). There was no significant difference in the mean RGS value between the 60-second model group and the 90-second model group on days 21 and 28 ($p = 1.000$ and $p = 0.900$). The histological pictures at compression durations of 60 and 90 seconds show severe damage on spinal cord continuity. There was no significant difference in the mean value of TNF-α between the duration compressions of 60 and 90 seconds ($p = 0.937$).

Conclusion: The use of Yasargil aneurysm clips with a duration of 60 and 90 seconds could produce a contusion–compression SCI model with expressions of neuropathic pain, locomotor function, histology, and pro-inflammatory cytokine.

Keywords: Spinal cord injury model, contusion–compression, neuropathic pain, locomotors, TNF-α.

This research received no external funding.

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Abstract 2591

The 4D Nucleome Program

Riccardo Calandrelli, *University of California-San Diego*

The NIH Common Fund's 4D Nucleome (4DN) program was originally launched in 2015 with its first phase (2015–2020), and it has now reached the third year of its second phase (2020–2025). The goal of the program is to study the three-dimensional organization of the nucleus in space and time (the 4th dimension), with its second phase focusing more towards addressing the role of nuclear organization in health and disease. The nucleus of a cell contains DNA, the genetic "blueprint" that encodes all the genes a living organism uses to produce proteins needed to carry out life-sustaining cellular functions. Scientists know that how the information in the nucleus is organized, stored, and unpackaged are all important to basic human health and we are only starting to learn how changes in this organization can lead to the development of different diseases, such as cancer, or our response to infectious agents like viruses. The 4DN program has generated a variety of tools and resources so scientists can continue to learn about the importance of nuclear organization. Program deliverables currently available through the public 4DN Portal (https://www.4dnucleome.org/) include nearly 2000 datasets from hundreds of experiments, 82 software packages, 11 protocols officially approved by the Steering Committee, and many reagents and biomaterials for researchers to use. Periodical web tutorials provide training on how to use 4DN software and tools. This training material is shared with the entire community on the 4DN YouTube channel, in addition to scientific webinars featuring novel studies and discoveries within the 4DN consortium. In this context, the 4DN Organizational Hub provides the administrative infrastructure necessary to facilitate and coordinate all the activities of the 4DN Network, both within and outside of the community.

The 4DN Organizational Hub is funded by the NIH Common Fund 4D Nucleome grant U01CA200147.

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Abstract 2636

Community Engaged Learning as part of Senior Research Seminar and Chemistry and Biochemistry Club Activities

Dennis Merat, *Christian Brothers University*

The Outreach Program involves Community -engaged Learning that seeks to involve both the classroom and the Christian Brothers University (CBU) biochemistry and chemistry clubs in our K-12 education focused activities. These activities include a regional science fair for middle and high school students, the Chemistry Olympiad local and national competitions, and a summer outreach program for K-5 children who were recent immigrants from war-torn areas of Africa. Students in a senior-level research seminar class had an opportunity to put their skills to use by assisting in the judging of research projects conducted by grades 6, 7, and 8 students in our middle school fair which was affiliated with the Broadcom Masters national competition. Students in both the biochemistry and chemistry clubs and the research class have assisted with both the middle school and high school fairs by setting up tables for the presenting students and serving as information guides for the middle and high school students and their parents and teachers who came to our CBU campus to participate in the fairs prior to the Covid-19 pandemic. The assistance of our students from the research course and the clubs has enabled us to allow area students from public, private, and home-school to compete in the fairs at no charge to them or their parents or schools. Students from the biochemistry and chemistry clubs also helped by serving as information guides at the Chemistry Olympiad competitions and preparing the lab for the practical experimental section of the National Chemistry Olympiad Exam. These programs are resuming this spring with In-person only fairs and Chemistry Olympiad competitions with active participation by our students. Also prior to the COVID-19 pandemic, students from the biochemistry and chemistry clubs participated in the summer enrichment programs outside CBU for children of recent immigrants from Africa. These programs focused on a variety of STEM related activities related to chemistry, weather, geology, and fractals.

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Abstract 2729

Utilizing Cultural Humility to Create Inclusive Laboratory Spaces

Antentor Hinton, *Vanderbilt University*

Cultural humility is a crucial skill for the science, technology, engineering, mathematics, and medicine (STEMM) field, for supporting persons excluded because of their ethnicity or race (PEERs). Critically, cultural humility allows a for a better mutual understanding and appreciation of others, through fostering positive interactions with different kinds of individuals. In academia, it is important to acquire cultural humility for individuals to improve their grit, focus, and goal outcomes, and also create a more robust mentor relationship. For instance, the qualities of a mentoring relationship may be dependent on the academic status of a mentee and remains mutual humility. A mentor must be able to respond to these needs with cultural humility. In this talk, I offer suggestions for becoming fully responsible for one's actions, how to utilize cultural humility to create a more inclusive laboratory, and offer solutions for creating and fostering an environment of greater cultural humility.

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Topic Category Signal Transduction and Cellular Regulation

Abstract 141

Role of TP53 codon 72 polymorphism in macrophage polarization

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The purpose of this study is to determine the role of the most common p53 single nucleotide polymorphism (SNP) at codon 72, which encodes proline (P72) or arginine (R72), in the regulation of macrophage polarization. A common single nucleotide polymorphism (SNP) in the tumor suppressor gene TP53 occurs at codon 72. There are two variants of this codon: a proline (P72) and an Arginine (R72). This polymorphism has been demonstrated to be geographically variable with P72 occurring in populations whose ancestry originates around the equator (in Africans at ~95% frequency), and the R72 allele occurring in population farther away from the equator (northern Europeans have ~80–85% arginine). Numerous studies have associated this polymorphism with differential risk for developing cancers, diabetes and metabolic diseases. However, the molecular mechanisms behind differential impact of these variants on cancer and metabolic disorders remain unexplored. Here, we show this polymorphism impacts macrophage polarization. To investigate the effects of this polymorphism on immune response, we used a Human p53 Knock In (Hupki) mouse derived bone marrow macrophages (BMDM), homozygous for either P72 or R72. Lipopolysaccharide (LPS) and IL-4 was used to activate macrophages. Signaling pathways involved in macrophage activation were analyzed by Real Time RT-PCR, Western blotting, and immunofluorescence. Volumes of tumors generated by subcutaneous injections of tumor cells (TC1), mixtures of tumor cells and LPS-stimulated P72 macrophages (TC1 + P72LPS), or mixture of tumor cells and LPS-stimulated R72 macrophages (TC1 + R72LPS) were measured every four day, with tumor volumes calculated as length × Width × Width/2. We found that macrophages that carry R72 are reluctant to become classically activated macrophages (M1) as demonstrated by the reduced expression of M1 genes (iNOS, IL-12 and Socs1) Vs P72 when stimulated with LPS. Likewise, reduced cytokines secretion (IFN-β and IL-12) by R72 BMDMs demonstrates its inability to respond inflammatory stimuli. Additionally, ability of P72 macrophages to M1 phenotype was driven by recruitment of NF-kβ and STAT1 on promoter of pro-inflammatory genes to initiate transcription. Data from mouse model of cancer confirm impact of this polymorphism on macrophage function *in vivo* by demonstrating that R72-LPS stimulated macrophages lose ability to reduce tumor growth. Our study reveals difference in immune response of P and R macrophages contributes to increased risk of cancer and metabolic diseases in R72 variant due to differential ability of polymorphism to macrophage polarization. In conclusion, we report that macrophages carrying R72 variant are biased toward M2 phenotype through altered NF-kB and STAT1 signaling which impact *in vivo* macrophage function as R72-LPS stimulated macrophages lose ability to reduce tumor growth.

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Abstract 1172

ATF4 Downstream Effects are Cell-Context Specific in Neurons and Glia

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Blake Jones, Austin Lewis, Sharmina Miller-Randolph, Jeffery Twiss, Pabitra Sahoo

The nervous system is composed of neurons and glial cells, with axons of neurons providing long-range communication between the neurons and their targets. In the peripheral nervous system (PNS), nerve injury activates new gene transcription in neurons that supports axon regeneration – activation of transcription factors is needed for the initial injury response and transition to a growth-associated transcriptome. The denervated glial cells in injured PNS nerves (Schwann cells [SC]) re-enter the cell cycle to transition from a myelination- to regeneration-promoting phenotype. Activating transcription factor 4 (ATF4) is an oxidative stress-induced transcription factor whose activation has been shown to trigger cell death. For example, axonally-synthesized ATF4 in response to Alzheimer's disease-causing Amyloid β peptide 1–42 has been shown to induce neurodegeneration. In contrast, other data link ATF4 to neurite growth and maturation. Using mixed cultures of adult dorsal root ganglion (DRG) that contain sensory neurons and PNS glial cells, we find distinct outcomes for ATF4 over-expression depending on the cell-context. ATF4 overexpression significantly increases axonal outgrowth from cultured sensory neurons, while simultaneously triggering death of SCs in the same cultures. Since SCs are known to support axon growth, it is surprising to see increased neuronal growth while the SCs are dying. We used RNA-seq to determine if ATF4 overexpression differentially affects the transcriptomes for growth promotion in the neurons vs. death in SCs. We find 1516 differentially expressed genes (DEG) in neurons vs 163 upregulated genes in SCs with ATF4 overexpression. RT-ddPCR validated increased expression of 4 genes in neurons and 3 genes in SCs and decreased expression of 2 genes in neurons with ATF4 overexpression. siRNA knockdown was used to test for sufficiency of these DEGs in growth-promotion vs. SC death in response to ATF4 overexpression. These mRNA depletion studies show that upregulation of Slc16a14 is sufficient for ATF4-dependent neuron growth promotion in sensory neurons, and upregulation of Cox6a2, Stc2, and Sfrp4 are each sufficient for ATF4-dependent SC death. Overall, these data indicate that ATF4 can either activate growth-promoting or cell death-promoting signaling cascades in a cell-context specific manner.

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Abstract 1174

Role of Phytoestrogens as an Anticancer Therapy Against Inflammatory Breast Cancer

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Keishla Rodríguez Mártir, Xavier Bittman, Esther Peterson

Inflammatory breast cancer (IBC) prevails as the most lethal form of breast cancer. The effectiveness of current treatment options and hormone therapies, however, is limited; IBC subtypes SUM149 and SUM190 are hormone receptor-negative, lacking expression of estrogen and progesterone receptors. Phytoestrogens—naturally derived structural analogs to 17β-estradiol (E2)—were evaluated as an alternative treatment option using 2D *in vitro* IBC models. Given the estrogenic properties of phytoestrogens, the drugs were hypothesized to interact with targets in pathways similar to E2 in non-genomic signaling. Previously documented responses of IBC to phytoestrogen treatment showed an increase or reduction in cell viability depending on the cell line and phytoestrogen used, therefore, both SUM149 and SUM190 subtypes were studied using monotherapy and combination methods with phytoestrogens including coumestrol (Cou), genistein (GN), and 8-prenylnaringenin (8-PN). Prior work has outlined the monotherapeutic effects of Cou and GN with SUM149 and SUM190, though little was known about the effects of the most potent phytoestrogen, 8-PN. Changes in cell viability were assessed using Alamar Blue viability assays; reductions in cell viability were observed following monotherapy treatments of Cou and GN in SUM149, while these treatments increased viability or had a varied effect in SUM190. RNA interference methods were used to suppress the over-expression of human epidermal growth factor receptor 2 (HER2) in SUM190 to analyze the role of HER2 in affecting the response of IBC cells to phytoestrogen treatment. Changes in the response of SUM190 to Cou following a partial HER2-knockdown can be observed, though complete sensitization was not achieved. Monotherapy treatments of 8-PN, however, reduced cell viability with SUM190 as well as with SUM149. Combinations at IC50 values of Cou and 8-PN, as well as Cou and GN, further decreased cell viability of SUM149 trials. Considering observed reductions of cell viability in SUM149 and SUM190—and the found effectiveness of 8-PN at stimulating such reductions—phytoestrogen treatment could serve as an alternative therapeutic strategy worthy of continued *in vitro* and *in vivo* study. This work offers insight into the effect of phytoestrogens on the oncogenic phenotypes of SUM149 and SUM190.

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Abstract 1180

Interleukin-33 expression is upregulated in colonic stem cells after irradiation

Mirika Jambudi, *The Pingry School*

Interleukin-33 (IL-33), a pleiotropic cytokine, plays critical roles in intestinal immunity, contributing to tissue homeostasis and responses to infection and inflammation. Extracellular IL-33 can be sequestered by its decoy receptor, soluble ST2 (sST2). While sST2 has been identified as a predictive biomarker of intestinal injury, including graft-versus-host disease and inflammatory bowel disease, the role of IL-33 in intestinal stem cell (ISC) compartment remains unclear. In colon, the ISC compartment consists of colonic stem cells necessary for epithelial regeneration upon damage and niche cells providing growth factors to the stem cells. Given the inability of traditional two-dimensional (2-D) imaging to precisely evaluate the IL-33-producing cell localization and its relationship to the ISC compartment, we sought to develop an approach using three-dimensional (3-D) microscopy of intact colonic tissue from IL33-GFP reporter mice following total body irradiation (TBI) to analyze the specific locations of IL-33-producing cells within colon after damage. Using this approach, we found that IL33-GFP+ cells were predominantly located at the submucosal layer and some IL33-GFP+ epithelial cells located at the crypt base during homeostasis. IL33-GFP+ epithelial cell number significantly increased in the colonic crypt base after TBI. To phenotype IL33-GFP+ epithelial cells in the colonic stem cell compartment at crypt base, Lgr5-GFP ISC reporter mice were used to establish an approach for evaluating the colonic stem cells and niche cells. Using Lgr5-GFP mice with whole-mount immunofluorescence staining, cKit immunostaining labeled Lgr5-GFP- colonic niche cells and Smoc2 immunostaining labeled Lgr5-GFP+ colonic stem cells in the stem cell compartment. Assessment of IL33-GFP+ epithelial cells in the stem cell compartment with immunostaining of cKit and Smoc2 demonstrated upregulation of IL-33 expression in Smoc2+ colonic stem cells after radiation injury. These findings suggested that IL-33 plays a role in colonic stem cells after intestinal damage.

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Abstract 1185

Effects of Cannabidiol in a *Caenorhabditis elegans* Amyotrophic Lateral Sclerosis Model

Juliet Sostena, *The Nueva School*

Molly Cavet, Albert Huang, Leilani Campos, Nicolas Burlinson, Ryan DeSantis, Luke De

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease caused by the progressive death of motor neurons. Cannabidiol, the second most prevalent cannabinoid in the *Cannabis sativa* plant, is a potential therapeutic tool for ALS due to its antioxidant, anti-inflammatory, and anti-spasticity effects, as well as its complementary role in treating other neurodegenerative diseases. In SOD1-G93A murine ALS models, cannabinoids have been shown to slow disease progression, extending lifespan and increasing motor function. However, the effects of specific cannabinoids—including cannabidiol—are yet undefined and their functions slowing disease progression are unknown. To advance this understanding we aim to study the effects of cannabidiol treatment in a *Caenorhabditis elegans* ALS model: a SOD-1 mutant transgenic strain with SOD-1 aggregation in muscular cells. We will use a death assay to measure the lifespan of SOD-1 mutant *C. elegans* and cannabidiol-treated SOD-1 mutant *C. elegans* to investigate whether treatment with cannabidiol impacts the lifespan of SOD-1 mutants. To assess locomotion, we will touch *C. elegans* with suture tips and use our novel computational analysis system to measure *C. elegans* movement response. We will then compare the motor response of SOD-1 mutant transgenic strain to wild type and study if cannabidiol modulates a possible change in motor response. This study will evaluate the functions of cannabidiol as a potential therapeutic tool in ALS using a SOD-1 mutant *C. elegans* model.

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Abstract 1186

Examining the Role of nmr-1 in *Caenorhabditis elegans* Behavioral Response to Antipsychotic Drugs as a Model for Glutamatergic Dysfunction in Schizophrenia

Lauren Stoffel, *The Nueva School*

Kyra Hong, Max Manning, Micah Brown, John Crown, Paul Hauser

Schizophrenia affects approximately 24 million people worldwide, and its incidence is rising. However, the heterogeneity of the disease's symptoms make it difficult to study. Studies have historically examined dopaminergic dysfunction, but recent findings indicate that glutamatergic dysregulation may also play a significant role in disease progression. The N-methyl-D-aspartate (NMDA) and NMDA receptor system in the glutamatergic pathway was first implicated via studies that showed NMDA receptor antagonists induced and exacerbated symptoms that resemble those present in schizophrenia. Specifically, mutations in the NMDA receptor 1 gene, Grin-1, have been associated with schizophrenia in genetic associativity studies, but the mechanism by which Grin-1 this relationship occurs is unknown. Thus, we propose to study the effects of Grin-1 via its homolog nmr-1 in the nematode *Caenorhabditis elegans*. The nematode *C. elegans* is an ideal model because it is easy to study the connection between its genetics, brains, and behavior. Other organisms are largely used to model positive symptoms of schizophrenia, whereas *C. elegans* is highly effective for modeling social interaction, learning, and memory impairment, which are understudied negative and cognitive symptoms of schizophrenia. Previous studies have linked the dysregulation of nmr-1 to schizophrenic phenotypes, but as far as we are aware, there are no studies regarding this gene's effect on response to treatment. We examine this relationship by evaluating nmr-1 knockdown mutants' response to the antipsychotic haloperidol in a variety of assays meant to isolate symptoms of schizophrenia: social feeding (sociality), locomotion (avolition), and memory and learning. We hypothesize that nmr-1 knockdown will decrease social feeding, locomotion, and memory and learning outcomes in worms treated with antipsychotics. Results from this study will help establish *C. elegans* as a model for schizophrenia, and more closely examine the role of nmr-1 in the progression of schizophrenia symptoms.

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Abstract 1207

Targeting Cyclin K in pancreatic cancer

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Jixin Dong, Yuanhong Chen

This study focuses on investigating the role of Cyclin K in pancreatic cancer growth and chemotherapeutic sensitivity, aiming to provide pre-clinical evidence for Cyclin K-targeted therapy in pancreatic cancer. Unlike other well-known cyclins, Cyclin K is largely understudied in cancers. Data extracted from the TCGA database indicated that Cyclin K was overexpressed in pancreatic ductal adenocarcinoma (PDAC) and associated with reduced overall survival. Consistently, western blotting showed that Cyclin K was abundantly expressed in human and mouse PDAC cell lines. By using a Tet-On inducible system, we established Cyclin K-depleted and Cyclin K-overexpressed cell lines to evaluate the function of Cyclin K in pancreatic cancer. The proliferation assay showed that Cyclin K depletion led to retarded PDAC cell proliferation, whereas Cyclin K overexpression boosted cell growth. We further confirmed that Cyclin K was required for pancreatic cancer growth *in vivo*. In addition, cell cycle analysis showed that Cyclin K deficiency resulted in G1-S arrest, while Cyclin K overexpression promoted G1-S progression. By using an RT2 cell cycler array, we identified CDC20 as an important target of Cyclin K that mediated Cyclin K-induced PDAC cell proliferation. To investigate the influence of Cyclin K on chemo-sensitivity, we treated the PDAC cell lines with two newly synthesized Cyclin K molecular glue degraders (HQ461 and NCT02). We found that these degraders specifically ablated Cyclin K and its cognate kinase CDK12 in a very efficient manner. Importantly, we demonstrated that Cyclin K abrogation, either by Cyclin K degrader or Cyclin K knockdown, rendered PDAC cells more sensitive to GemTaxol (gemcitabine plus Taxol) treatment and PARP inhibitors (olaparib or niraparib), as indicated by increased cleavage of PARP or caspase 3. Together, our current results suggest: 1. Cyclin K promotes cell proliferation and G1-S transition in pancreatic cancer; 2. CDC20 mediates the function of Cyclin K on pancreatic cancer cell proliferation; 3. Cyclin K depletion synergizes with GemTaxol or PARP inhibitor in pancreatic cancer treatment. Our study revealed for the first time that Cyclin K plays an essential role in pancreatic cancer growth and chemo-sensitivity, and targeting Cyclin K may offer a great therapeutic avenue for pancreatic cancer patients.

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ASBMB

Abstract 1214

APEX1 (1–20) and Selenoprotein T protect endothelial cells against LPS-induced activation and apoptosis

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Ptok Johannes, Jakobs Philipp, Jan Greulich, Florian Von Ameln, Schaal Heiner, Ale-Agha Niloofar, Joachim Altschmied, Judith Haendeler

Sepsis is a life-threatening event caused by an exaggerated immune response upon infection with lipopolysaccharide (LPS) as the main causative agent. LPS can cause activation and apoptosis of endothelial cells (EC) leading to endothelial dysfunction, organ dysfunction and finally organ failure. Sepsis is responsible for approximately 11 million deaths per year worldwide with no effective therapeutic option available after a certain stage is reached. By protecting the endothelium, it might be possible to prevent pathological progression. Apurinic/apyrimidinic endodeoxyribonuclease 1 (APEX1) is a multifunctional protein with two different domains responsible for DNA repair and reduction of oxidized proteins. In preliminary work we demonstrated that the first twenty amino acids of APEX1 called APEX1 (1–20) are sufficient to inhibit EC apoptosis induced by oxidative stress. Thus, it is tempting to speculate that APEX1 (1–20) could protect EC from LPS-induced activation and apoptosis. Therefore, we transduced EC with a lentiviral expression vector for APEX1 (1–20) or an empty control vector and treated with LPS. To identify candidate genes upregulated in LPS-treated and APEX1 (1–20) expressing ECs we used RNA-sequencing and bioinformatic analysis followed by real-time PCR for validation. Expression of the selenoprotein T (SELENOT) gene was highly upregulated in both approaches, making it an interesting candidate for further evaluation in prevention of activation and apoptosis of EC. To study the impact of SELENOT on LPS-induced EC activation and apoptosis, we generated an expression vector with a N-terminal FLAG tag. EC were transfected with this vector and the localization of FLAG-tagged SELENOT in the endoplasmic reticulum was verified by immunostaining. Overexpression of SELENOT inhibited LPS-induced activation of the endothelium measured by intercellular adhesion molecule 1 (ICAM1) staining, and apoptosis was reduced. Thus, SELENOT, a target induced by APEX1 (1–20), has a protective effect in EC after LPS treatment. To further examine the effect of APEX1 (1–20) on LPS-induced endothelial dysfunction *in vivo*, we used C57BL/6 mice. After intraperitoneal LPS injection the mice were given APEX1 (1–20) peptide into the tail vein. To determine EC activation, ICAM1 expression in lung and liver tissue was investigated by real-time PCR. Mice treated with the APEX1 (1–20) peptide showed significantly lower ICAM1 expression after LPS treatment compared to LPS alone. Lastly, we also investigated whether low doses of LPS induce senescence in EC, since senescent EC exhibit a proinflammatory phenotype shown by elevated ICAM1 expression. As this was not studied before, we adapted our established protocol for H2O2-triggered stress-induced senescence for the LPS treatment. We incubated EC with LPS (1 ng/ml) every second day for two weeks. We found increased nuclear p21 staining as a marker for senescence in EC. Thus, this model could be suitable to determine whether APEX1 (1–20) could also delay or prevent senescence induction. Taken together, APEX1 (1–20) and SELENOT may provide potential therapeutic options to prevent LPS-induced endothelial dysfunction.

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Abstract 1231

Deletion of Mdm1 induces retinal degeneration due to intraflagellar transport defects of photoreceptor cells

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Mouse double minute 1 (Mdm1) might be involved in the function and structure of centrioles and age-related retinal degeneration. However, the mechanism by which Mdm1 deficiency causes retinal degeneration remains unknown. We confirmed that the Mdm1 protein is localized at the connecting cilium (CC) of photoreceptor cells in the retina. The electroretinograms of 6-week-old Mdm1$^{-/-}$ mice revealed decreased vision, which was eventually lost, and outer segment (OS) photoreceptor degeneration was evident on postnatal day 7, with complete loss of the outer nuclear layer (ONL) observed at 35 weeks. Mdm1$^{-/-}$ mouse retinas showed mislocalization of opsins in the photoreceptor cells, indicating particular intraflagellar transport (IFT) defects, and entrapment of the nuclei in the ONL by microvilli of retinal pigment epithelial cells, leading to apoptosis in the ONL. These results suggest that Mdm1 ablation causes specific IFT defects, which prevents the OS from continuously replenishing new discs, resulting in retinal degeneration.

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Abstract 1235

Role of pericardial cells in myocardial infarction

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Rianne Nederlof, Andre Heinen, Stefanie Goedecke, Axel Godecke

Epicardium is a single-cell layer of mesothelial origin, enveloping the heart as a visceral layer of the serous pericardium. During the fetal stage epicardium serves as a source of progenitor cells and promotes a paracrine milieu, critical for myocardial growth and vascular patterning. One of the most essential epicardial lineage markers, Wt1, is highly expressed at this period; however, in the adulthood, under homeostatic conditions, the epicardium becomes quiescent and Wt1 expression diminishes significantly. Interestingly, in mice exposed to a cardiac ischemia, the epicardial layer thickens near the injury and Wt1 expression substantially increases, rather throughout the entire epicardium. Lineage tracing demonstrated that re-activated epicardial Wt1+ cells contribute to fibroblast or endothelial cells lineages and secret paracrine factors such as FGF2 and VEGFA. Although the epicardium has been intensively investigated within the last decades, its adjacent parietal and fibrous pericardial layers, surrounding the heart and the roots of great vessels, were in a minor focus. Therefore, we aimed to examine the outer layer, its cell composition and response to myocardial infarction (MI). Histological analysis demonstrated that in comparison to the dormant epicardium, the pericardium contains a high number of Wt1 expressing cells already under basic conditions. The observation was confirmed by the lineage-tracing model Wt1CreERT2;Rosa26Tomato mice which express RFP in Wt1+ cells in a tamoxifen inducible manner. According to qPCR analysis, the pericardial Wt1 gene expression was 10-fold higher than in cardiac tissue (n = 5). In addition to Wt1+ cells, the pericardium and its fat depot contained CD68+, F4/80+ macrophages and CD19+ B-cells. After 5 days in culture, the isolated pericardial cells expressed Wt1 and Periostin, a marker of activated fibroblasts. According to FACS analysis, the total amount of pericardial cells under basic conditions was 80218 ± 17428 cells per pericardium (c/p), among them CD45+ macrophages and B-cells (36040 ± 6371 c/p), CD31+ endothelial cells (2944 ± 2036 c/p), and MEFSK4+ fibroblasts (11687 ± 5460 c/p). To examine a pericardial response to MI we have implemented a murine model of MI by ligating LAD with closed pericardium and analyzed the tissue on day 6 after the surgery. According to the histological analysis, the pericardial thickness increased from 10 ± 3 μm in control mice up to 147 ± 45 μm (the scar vicinity) and 17 ± 14 μm (the remote area) in mice after MI surgery. However, in sham experiments the pericardial thickness also increased up to 113 ± 14 μm and 12 ± 0.3 μm along left and right ventricle respectively. Moreover, under MI conditions the parietal pericardium contained adipocytes, integrated in the fibrous layer and fat-associated lymphoid clusters (FALC)

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consisting of Wt1+, CD68+, CD31+ and CD19+ cells and located predominantly along the scar. We have shown that the pericardium is composed of immune cells, fibroblasts and Wt1+ cells, of which the latter might represent a set of progenitor cells. The pericardium can be activated upon damage suggesting that it may substantially modulate cardiac diseases.

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Abstract 1248

pH selective reactions to selectively limit cancer cell proliferation: Effect of CaS nanostructures in skin human melanoma and benign fibroblasts

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Olga Rodriguez Martinez, Amy Wu Wu, Carolina Colon

Cancer continues to be a global health issue that challenges the scientific community. New developments still encounter setbacks that range from therapy resistance and lack of response to severe secondary effects. An acidic extracellular pHe value is characteristic of many cancers, in contrast to the basic physiologic extracellular pHe found in most benign cells. This difference in pH is a unique opportunity to design chemicals that can be employed for pH selective reactions in the extracellular fluid of cancer cells. CaS nanoclusters will dissociate into Ca2+ ions and H2S in acidic environments only and has potential to become an active ingredient to selectively treat cancer cells. The viability of human skin melanoma cells and corresponding benign fibroblasts exposed to dispersions containing CaS nanoclusters is reported. The viability of melanoma cells decreases with dispersion concentration and reaches 57%, a value easily distinguishable from the melanoma control experiments. In contrast, the viability of benign fibroblasts remains nearly constant within experimental error over the range of dispersion concentration studied. Fluorescence microscopy measurements reveal that the CaS dispersions facilitate vinculin delocalization in the cytoplasmic fluid a result consistent with improved FAK regulation in treated melanoma cells. The concentration of caspases 3 and 9 is found to be significantly higher in treated melanoma cells than in the corresponding controls, consistent with the activation of intrinsic apoptotic mechanisms. No statistically significant difference is found in the concentration of caspase 8 between the treated and control melanoma cells. The selectivity of the CaS dispersion is proposed to result from the acidic extracellular pH of melanoma cells- as opposed to the benign fibroblasts- that facilitates the formation of Ca2+ ions and H2S which can in turn activate apoptotic mechanisms facilitated by FAK activation. The results are discussed in the context of the potential biomedical applications of CaS dispersions in cancer therapies by facilitating FAK regulation and/or selective apoptosis.

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Abstract 1249

14-3-3 binding stabilizes and sequesters PTOV1 to the cytoplasm during G1

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Cells must respond to changing environments by modulating signaling and gene expression patterns to maintain homeostasis, health, and viability. Inappropriate responses lead to disease processes, such as cancer. Despite improvements in targeted treatment options, cancer remains the second leading cause of death in the United States, illustrating the need to develop new highly effective targeted therapies. 14-3-3 interacts with a network of phosphorylated proteins to support signaling pathways promoting oncogenesis, metastasis, growth, cellular survival, and chemoresistance in a variety of cancers, including lung, prostate, and breast. We recently identified prostate tumor overexpressed 1 (PTOV1) as a novel 14-3-3 interacting protein promoting tumorigenic gene expression patterns. We showed that 14-3-3 binding sequesters PTOV1 in the cytoplasm and protects PTOV1 from proteasomal degradation. Using double thymidine block synchronization, we now demonstrate that both PTOV1 S36 phosphorylation and total PTOV1 protein levels peak in G1, prior to PTOV1 translocating into the nucleus during S phase. These data support our model that phospho-dependent 14-3-3-PTOV1 binding maintains PTOV1 in the cytoplasm during G1, where PTOV1 is stabilized to carry out translational regulation, including promoting cJun expression. As the cell enters S phase, loss of phosphorylation and the accompanying loss of 14-3-3 binding allows PTOV1 to translocate into the nucleus, carry out relevant nuclear gene expression functions, and then be degraded via the proteasome.

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Abstract 1250

Disrupting the Transmembrane-Mediated Oligomerization of the Receptor Protein Tyrosine Phosphatase JPromotes its Activity

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Receptor Protein Tyrosine Phosphatases (RPTPs) are one of the most important regulators of receptor tyrosine kinases (RTKs) and play a crucial role in mammalian signal transduction. But our still-incomplete understanding of the structural aspects of their regulation and the lack of selective agonists have hampered efforts to understand their roles in downstream signaling regulation and to pursue them as potential therapeutic targets. However, the reported ability of RPTP homodimerization to antagonize their catalytic activity presents potential opportunities to develop unique strategies to modulate the activity of RPTPs. We reported that the homodimerization of a representative member of the RPTP family (protein tyrosine phosphatase receptor J or PTPRJ; also known as DEP1) is regulated by specific transmembrane (TM) residues and that these interactions are essential in regulating its enzymatic activity and substrate access in cells. For instance, disrupting PTPRJ TM-mediated oligomerization through point mutations counteracts the activity of EGFR and FLT3 in cells. Building on these insights, we report here the design and testing of a tumor-selective peptide capable of binding to the TM domain of PTPRJ and disrupting its homodimerization. Doing so promotes PTPRJ TM-mediated access to EGFR, reduces the phosphorylation of EGFR (a known substrate) and other downstream signaling effectors, and antagonizes EGFR-driven cancer cell phenotypes. This first-in-class agent represents a possibly orthogonal way to target the activity of PTPRJ and RTKs phosphorylation. It could therefore be used as probes to tease out PTPRJ regulating mechanisms and for therapeutic purposes via the attenuation of signaling by dysregulated RTKs in cancers. We also expect that the basic framework developed here can be extended to other RPTPs.

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Abstract 1260

Eukaryotic cell death is mediated by arginyltransferase1 by a mitochondria-dependent pathway

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The regulation of cell death in eukaryotic cells is essential for stress adaptation and signal response. Recent studies showed that stress-induced cell death events is dependent on the activity of arginyltransferase1 (ATE1), an evolutionarily conserved enzyme that induces the degradation of many proteins via a posttranslational modification called arginylation. However, exactly how ATE1 achieves this role remains largely unknown. Here, by using budding yeast as a test model, we found that cytosolic ATE1 is relocated into mitochondria under oxidative stress. We also found that this translocation of ATE1 is essential for the initiation of apoptosis. Furthermore, we found that the ATE1-mediated cell death is not dependent on mitochondrial ETC activity or the classic caspase pathway. Rather, we found that ATE1-mediated cell death is dependent on the formation of mitochondrial permeability pore and the release of apoptosis-inducing factor. We conclude that ATE1 is a novel apoptosis-regulator that utilizes specific mitochondrial pathway.

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Abstract 1262

Histamine activates human microglial cells and increases prion protein expression; A role for prion protein in neuroinflammation?

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Background: Microglia are the most prominent brain immune cell, and in their activated state, these cells can either be neuroprotective, protecting the brain against tissue injury, or destructive, promoting synaptic loss in neurodegenerative disease. In the brain, microglia can be activated by the peripheral inflammatory mediator histamine, which is physiologically released by brain mast cells during degranulation. The consequence of histamine activation of microglia has not been well-studied, but there is evidence that histamine may alter microglial protein expression such as prion protein (PrPC) expression. Although the physiological role of PrPC is not well understood, this protein is found in many cells, including microglia, and studies have demonstrated that brain cells may change their expression of PrPC during disease indicating a potential role of PrPC in the regulation of immune cells.

Objective: The aims of this study were to examine whether histamine induces human microglial cell activation and to measure levels of total and surface PrPC expression in HMC3 cells before and after activation with histamine.

Methods: The human microglial cell line (HMC3) was used as a model. Surface and total PrPC protein expression levels were measured using flow cytometry and western blot analysis, using antibodies that recognize different epitopes on PrPC. Histamine effects on metabolic activity were measured using XTT assay. qPCR was used to identify expression of histamine receptor mRNA. Cytokine and chemokine production were measured by ELISA.

Results: Human microglial clone 3 (HMC3) cells express mRNA for at least three of the four histamine receptors (H1R, H3R and H4R), with mRNA expression being at least two-fold higher than GAPDH controls. Resting HMC3 cells constitutively produced both IL-6 and IL-8, but histamine (100 μM for 24 hours) activated HMC3 cells increased their production of IL-6 (193.9 ± 3.689 pg/mL) compared to untreated cells (122.2 ± 0.8717 pg/mL; $n = 3$, $p < 0.0001$) and IL-8 (92.78 ± 1.667 pg/mL) compared to untreated cells (60.72 ± 1.097 pg/mL; $n = 3$, $p < 0.0001$). Flow cytometry and western blot analysis indicated that HMC3 cells expressed relatively large amounts of PrPC (compared to SHSY5Y cells), and more than 98% of HMC3 were positive for surface PrPC expression. Western blot analysis of HMC3 lysates showed a strong band at 37 kDa, indicative of di-glycosylated PrPC, but also showed a glycosylated PrPC isoform at 26 kDa and multimeric PrPC at 125 kDa. Furthermore, histamine treatment increased surface PrPC expression (100 μM for 24 hours; MFI = 1383 ± 72) compared to untreated cells (MFI = 1169 ± 95).

Conclusions: Although previous research has examined the effect of histamine on murine microglial models, our data shows, for the first time, that human microglia express histamine receptors and are activated by histamine to produce key inflammatory mediators. We have also made the novel observation that histamine upregulates expression of PrPC on human microglia, suggesting that local production of histamine in the brain may have profound implications for PrPC function. The mechanisms that control PrPC expression are still poorly understood and, to our knowledge, this is the first demonstration that PrPC expression can be modulated by a proinflammatory mediator such as histamine. Since PrPC misfolding is a common feature of brain disease, our observations may have far-reaching implications for neuromodulation of prion-mediated neuropathy.

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Abstract 1264

High-resolution structures using cryo-EM guide drug development for Neuromyelitis Optica

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Neuromyelitis Optica (NMO) is an autoimmune disease where human antibodies self-target the water channel AQP4. Pathogenic AQP4 autoantibodies (AQP4-IgG) bind to hAQP4 on CNS astrocytes and initiate tissue injury and neurologic impairment through both lytic and non-lytic mechanisms. AQP4-IgG mediated effector functions (complement-mediated cytotoxicity [CDC] and antibody-dependent cell-mediated cytotoxicity (ADCC) cause targeted astrocyte lysis, promote immune cell infiltration, and propel secondary myelinolysis, axonal injury, and neuronal destruction. AQP4-IgG recognizes conformational epitopes formed by the extracellular loops (Loops A, C, E) of hAQP4 tetramers or higher-order orthogonal arrays of protein (OAPs). The three-dimensional structure of AQP4-IgG epitopes are likely to play an important role in dictating CNS injury by modulating antibody effector function and astrocytic activity. Bennett lab has previously demonstrated that AQP4 rAbs sensitive to C loop mutations at H151 and L154 show enhanced complement activation on hAQP4 OAPs. We determined molecular structures of AQP4 apo and AQP4 bound to patient derived autoantibodies in nanodiscs using cryo-EM. Our data provides high-resolution details of the interface between AQP4 and autoantibodies for drug designing purposes. Here, we highlight the structural differences in the way these antibodies bind and hence dissecting the mechanism of immune responses generated to develop a better drug-development platform. These are the first structures of a channel demonstrating an autoimmune disease.

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Abstract 1280

Cysteine phosphorylation controls magnesium homeostasis through the PRL/CNNM pathway

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The PRLs (Phosphatases of Regenerating Liver) are members of the dual-specificity subfamily of protein phosphatases and implicated in tumor growth and metastasis. The three members are highly oncogenic and overexpressed in many cancers, yet their mechanism of action remains unknown. Recently published studies have suggested that PRL oncogenicity is mediated through the activity of a family of magnesium transporters, CNNM. Like other cysteine-based phosphatases, catalysis is mediated by a conserved, catalytic cysteine but PRLs are unusual in that the phosphocysteine intermediate is very long lived. Here, we use biochemical and structural studies to show that the interaction between PRLs and CNNMs is regulated by cysteine phosphorylation and oxidation. *In vivo*, PRLs are endogenously phosphorylated on cysteine to high levels and this phosphorylation changes in response to magnesium levels. The X-ray crystal structure of the PRL-CNNM complex reveals the molecular basis for regulation by cysteine phosphorylation. These studies suggest PRLs function both as pseudophosphatases and true phosphatases to regulate magnesium homeostasis and tumor metastasis.

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Abstract 1286

Targeting PERK/ATF4/LAMP3 with folate deprivation impedes cancer hallmarks in liver cancer cells

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Cancer cells generally demand increased protein synthesis and folding. This raised requirement for protein folding beyond folding capacities leads to ER stress which subsequently activates ER stress sensors: IRE1, ATF4, and PERK. Folate deficiency is already known to escalate ER stress, likely through the accumulation of homocysteine. Previous results from the lab showed an increased expression of ATF4 in HCC cells in folate deficiency conditions. In the present study, we determined the role of folate deficiency in ER stress and the effect of ER stress inhibition in hepatocellular carcinoma cells. To achieve this, HepG2 cells were cultured in both folate normal (FN) and folate deficient (FD) conditions. ER stress pathway genes were studied using RT-qPCR. To determine the effect of FD on cancer, cancer hallmarks i.e. migration and invasion were performed by trans-well assay, metastasis and cancer stem cell-like phenotype by sphere formation assay, epithelial to mesenchymal transition (EMT) by gene expression (RT-qPCR) of epithelial markers (syndecan and cadherin) and mesenchymal marker (vimentin) and the effect on apoptosis was measured by Annexin-PI assay using flow cytometry. We found that FD led to the activation of UPR via induction of PERK/ATF4/LAMP3 only in HepG2 cells. FD treatment in HepG2 cells also reduced the migratory capacity and the invasiveness of HCC cells and reduced mesenchymal marker vimentin with an increase in the expression of syndecan and cadherin but induced apoptotic cell death. Treatment with GSK2606414 (PERK inhibitor) decreased the FD-induced expression of PERK, ATF4, and LAMP3 in FD cells. Also, GSK2606414 was found to increase apoptotic cell death and to further reduce the cancer hallmarks with a decline in the cancer stem cell-like phenotype along with invasiveness and migratory capacity selectively in FD cells and not in FN cells. Not only this, inhibition of PERK/ATF4/LAMP3 arm induced the expression of epithelial markers with a reduction in expression of vimentin. This phenomenon was specific to HCC cells as these changes were not there in A549 (lung carcinoma) and Cal-27 (oral squamous cell carcinoma) cells exposed to FD and treated with GSK2606414. Altogether, our data suggest that targeting the ER stress pathway along with folate depletion may provide a promising strategy for the elimination of metastatic potential of HCC cells & thus contributing to more effective therapeutic options.

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Abstract 1287

Early Changes in N-Methyl-D-Aspartate Receptor Subunits in the Development of the 5xFAD Alzheimer's Mouse Model

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Alzheimer's disease (AD) is an incurable brain disease that is the most common form of dementia. There is a critical need to design treatments that can intervene in early events in the development of AD in order to prevent or delay the onset of the disease. Our recent findings showed that the 5xFAD mouse model, which was believed to develop synaptic dysfunction only at 6 months of age, actually showed synaptic alterations in N-methyl-D-aspartate receptor (NMDARs) subunit responses by 0.5–4 months of age, which could lead to alterations in memory. The 5xFAD mouse model has five mutations that are linked to familial (inherited) AD. We hypothesized that early changes in NMDAR synaptic responses in 5xFAD mutants are related to alterations in NMDAR protein expression. Based on our electrophysiological data that suggested changes in NMDAR function occur during development, we used brains obtained from a variety of ages (1–2 days and 0.5. 1, 2, 3, and 4 months) of wildtype and 5xFAD heterozygous male and female mice to examine the synaptic and extrasynaptic protein expression of NMDAR subunits in the hippocampus with the use of semi-quantitative Western blots. After analyzing males and females separately, we found that 5xFAD males showed higher overall expression of GluN2B in the hippocampal synaptic region than their wildtype counterparts, while 5xFAD females showed lower overall expression of GluN2B. 1–2 day old males showed lower overall expression of GluN2B in the hippocampal crude synaptosomes. This suggests that amyloid overexpression affected NMDA receptor expression and had different effects on males and females. In addition, the effects appeared to change across early development in males.

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Abstract 1290

Basal expression of nuclear transcription factor, NR4A1, is lower in triple negative breast cancer compared to other breast cancer subsets

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Background: Treatment of patients with triple-negative breast cancer (TNBC) has been challenging due to the absence of well-defined molecular targets and high invasive and proliferative capacities of these cells. Therefore, new therapeutic strategies for treatment of TNBC are urgently needed. NR4A1 (nuclear receptor subfamily 4A1), a nuclear receptor, acts as transcription factor and has been shown to play key role in breast cancer biology.

Methods: Clinical data were extracted from the METABRIC and TCGA breast cancer projects, hosted at the TCGA database. Q-RT-PCR and Western blotting were performed to visualize gene and protein expression. Immunohistochemistry of tumor microarray slides containing Triple negative breast Cancer (fifty cases/one hundred and fifty cores), Luminal A breast cancer (fifty cases/one hundred and fifty cores), and Luminal B breast cancer (one hundred fifty cases/one hundred fifty cores) were analyzed for NR4A1 expression as well. Data were graphed and analyzed using Graph Pad Prism Software 8 using one-way ANOVA and the unpaired two-tailed Students t-test. All data were evaluated in triplicate against vector control cells.

Results: Western blot analysis showed increased NR4A1 expression across the luminal A, luminal B, and HER2+ breast cancer subtypes, compared to the TNBC cell lines. T47D (luminal A subtype) exhibited the highest expression, MCF-7 (luminal A subtype) and BT-474 (luminal B) both showed increased expression as well. SKBR3 (HER2+) showed lower expression compared to the luminal subtypes but was significantly higher than most of the TNBC cell lines, outside of the MD-MB-468 triple negative breast cancer line. Quantitative polymerase chain reaction data revealed elevated mRNA expression in MCF7 (luminal A subtype), BT474 (luminal B subtype), and SKBR3 (HER2+ subtype) as compared to most of the triple negative breast cancer cells with the exceptions of SUM149PT and MFM-223 cells. Immunohistochemistry analysis also showed a significant down regulation of NR4A1 in tumor microarrays which contained luminal tissues as compared to TNBC tissues.

Conclusions: Breast cancer is a diverse and intricate disease which is known to have unique inter- and intra-tumoral characteristics. TNBC is refractory to clinically approved therapies due to increased therapy resistance as well as it's highly metastatic capabilities. NR4A1 has been shown to regulate pathways in proliferation, apoptosis, utilization of

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glucose, immune response, and DNA repair mechanisms within the human body. Understanding the differential gene expression of NR4A1 across these subtypes can serve as the fundamental basis for developing a therapeutic treatment. In addition, further in-depth investigations are required to evaluate the exact molecular mechanisms of NR4A1 in TNBC *in vivo*.

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Abstract 1294

Cancer-associated missense mutations in the tumor suppressor Polybromo-1 variably affect protein stability and function

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The chromatin remodeler and tumor suppressor Polybromo-1 (PBRM1) employs six bromodomains to bind acetylated histones and nucleic acids to modulate chromatin accessibility and gene transcription in health and disease. PBRM1 mutations are present in ∼40% of clear cell renal cell carcinoma (ccRCC) cases. Most PBRM1 mutations lead to complete loss of protein expression and tumor suppressor activity. However, missense mutations, which cluster within PBRM1 bromodomains, exist in ∼15% of ccRCC cases and ∼34% of all cancer cases. Such mutations generate full-length variant proteins with structural and functional characteristics yet to be elucidated. Here, we determined the biophysical and cellular effects of cancer-associated missense mutations across all six PBRM1 bromodomains. We employed sequence-based bioinformatics tools to define the mutational landscape of PBRM1 cancer-associated mutations. We show that missense mutations in PBRM1-BD2/BD4, the bromodomains most important for PBRM1-chromatin interactions, form a substantial component of this landscape. To computationally predict the effects of cancer-associated PBRM1 bromodomain mutations on protein structure and function, we complemented our bioinformatics analyses with in silico calculations of protein stability, global structural perturbations, molecular dynamics fluctuations, and ligand-binding capacity. We reveal that cancer-associated PBRM1 bromodomain mutations can be computationally classified as benign or deleterious. Additionally, deleterious missense mutations can be further characterized as structural variants (SVs) that affect protein stability, dynamic variants (DVs) that affect ligand binding, or structural & dynamic variants (SDVs) that affect both parameters. We selected the most potentially deleterious PBRM1-BD2/BD4 missense mutations for further biophysical and functional analysis with differential scanning fluorimetry, AlphaScreen biophysical binding assays, circular dichroism, electrophoretic mobility shift assays, and cellular histone binding assays. We demonstrate that cancer-associated PBRM1-BD2/4 missense mutations variably decrease bromodomain stability and ability to bind acetylated histones and/or Widom 601 DNA. The magnitude of such effects is based on residue conservation and proximity to bromodomain ligand binding sites. Taken together, we establish that individual missense mutations in PBRM1-BD2/BD4 have unique impacts on bromodomain structure and function. This knowledge will enable future studies correlating specific cancer-associated PBRM1 mutations with the overall tumor-suppressive role of the protein,

thereby informing future personalized medicine approaches in the context of cancer.

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Abstract 1302

Protein supersaturation powers innate immunity and programmed cell death

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Our cells respond rapidly and decisively to tiny stimuli that could otherwise prove catastrophic, such as single molecules of viral RNA. We lack a thermodynamic understanding for how such sensitive decisions can be made with such limited information. To provide that understanding, we used in-cell biophysical approaches such as Distributed Amphifluoric FRET (DAmFRET) to dissect the mechanism of decision making by large protein assemblies known as innate immunity signalosomes. Focusing first on the CARD-BCL10-MALT1 (CBM) signalosome that governs NF-κB activation, we found that the sensitivity and executive function of the complex derive from a nucleation barrier to a disorder-to-order phase transition by the adaptor protein BCL10. Using optogenetic tools and single-cell transcriptional reporters, we discovered that endogenous BCL10 is functionally supersaturated with respect to the ordered phase even in unstimulated human cells, and this results in a predetermined response to stimulation upon nucleation by activated CARD multimers. Remarkably, the material structure of the BCL10 assemblies did not matter for activity, implying that signalosome structure has evolved via selection for kinetic rather than equilibrium properties of the constituent proteins. We next extended our biophysical analysis to over one hundred other protein domains in human innate immunity signaling, uncovering dozens that control signaling via sequence-encoded nucleation barriers. This widespread kinetic control over cell fate suggests that cells are literally waiting to die – pyroptosis, necroptosis, and alternative cell fates downstream of these proteins are thermodynamically favored, and therefore inevitable with time. Our findings therefore reveal a physical basis for the progressive nature of age-associated inflammation.

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Abstract 1310

Regulation of IFNγ-Stimulated Gene Expression in Macrophages by the Transcriptional Coregulator CITED1

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Macrophages are tissue-associated innate immune cells that are amongst the first responders to microbial infection. The ability of these cells to efficiently ingest and destroy pathogens is strongly influenced by their polarization state. When stimulated by interferon-gamma (IFNγ), macrophages become M1-polarized, increasing their production of proinflammatory cytokines and microbicidal reactive nitrogen species. Underlying this change in phenotype is the altered expression of >1000 interferon-stimulated genes (ISGs), largely regulated by signal transducer and activator of transcription 1 (STAT1) and interferon regulatory factor 1 (IRF1). The activity of these transcription factors must be carefully controlled to ensure that the response is sufficiently vigorous to resolve the infection but is spatially and temporally restricted to prevent prolonged inflammation and tissue damage. This is control is provided by a variety of cell-extrinsic and -intrinsic factors, including CBP/p300-interacting transactivator with glutamic acid/aspartic acid-rich carboxyl-terminal domain 2 (CITED2), which is constitutively expressed in macrophages and operates as a corepressor, reducing the ability of STAT1 and IRF1 to recruit CBP/p300 to ISG enhancers. We now show that macrophages exposed to IFNγ for over 24 hours express CITED1, another member of the CITED family of transcriptional co-regulators. Given its high degree of homology with CITED2, we hypothesized that CITED1 would also regulate ISG expression. To investigate this, we employed a gain- and loss-of-function approach by over-expressing or knocking out CITED1 in RAW264.7 murine macrophages and measuring changes in gene expression using RNA-sequencing-based transcriptome profiling. This was coupled with gene set enrichment analysis (GSEA) to quantitatively assess the impact of CITED1 on the transcriptional response to IFNγ stimulation and the expression of STAT1-regulated genes. Here, we find that CITED1 has the opposite effect to CITED2, functioning as a positive regulator of STAT1-dependent gene expression and enhancing the expression of multiple members of gene families associated with the IFNγ response. These include members of the C-C motif chemokine ligand (Ccl; Ccl2, Ccl3, Ccl4, and Ccl7), interferon induced protein with tetratricopeptide repeats (Ifit1, Ifit3, and Ifit3b), and Isg (Isg15 and Isg20) gene families. Additionally, we find that CITED1 activity is regulated at a transcriptional level and through cytosolic sequestration of the protein. The Cited1 promoter was scanned for transcription factor binding sites using the Eukaryotic Promoter Database and was found to have three STAT1 and two IRF1 putative cis-regulatory sites. Furthermore, IFNγ-stimulated expression of the gene was lost in STAT1 KO macrophages, indicating that Cited1 is itself an ISG. Finally, while CITED1 is exclusively cytosolic in unstimulated macrophages, IFNγ promotes the phosphorylation and nuclear accumulation of the protein. Collectively, our data indicate that CITED1 provides delayed positive feedback in the IFNγ-STAT1 pathway that enhances the expression of select ISGs in macrophages exposed to IFNγ for prolonged periods. Given the antagonistic effects of CITED1 and 2 on STAT1-regulated gene expression, we also propose that the changing ratio of these two proteins will dynamically adjust ISG expression and the M1 phenotype over the course of the IFNγ response.

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Abstract 1317

Interaction of the scaffolding protein IQGAP1 with elements of the MAP kinase and PI 3-kinase pathways: direct binding of the WW domain to p110alpha, and delocalized, cooperative binding of the IQ domain to ERK and MEK

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Recently there has been renewed enthusiasm for targeting protein-protein interactions as a therapeutic strategy for treating cancer and other diseases, and for using peptide-based inhibitors as drugs or drug leads in such approaches. Signaling scaffold proteins – large, multidomain proteins that bind to multiple members of one or more signaling pathways – participate in numerous protein-protein interactions that could be targeted to disrupt signaling. Indeed, studies using the human IQGAP1 scaffold protein have led to the discovery of two different small protein-protein interaction domains that, when engineered as cell-penetrating peptides, show broad anti-tumor activity with minimal associated toxicity. The first of these is the 27-residue WW domain of IQGAP1. Identification of a binding partner for this domain has proven elusive. We have now found that the WW domain of human IQGAP1 binds directly to the p110alpha catalytic subunit of phosphoinositide 3-kinase (PI3K). In contrast, the WW domain does not bind to ERK1/2, MEK1/2, or the p85alpha regulatory subunit of PI3K when p85 is expressed alone. However, the WW domain is able to bind to the p110/p85 heterodimer when both subunits are co-expressed, as well as to the mutationally activated p110/p65 heterodimer. We present a model of the structure of the IQGAP1 WW domain, and experimentally identify key residues in the hydrophobic core and beta strands of the WW domain that are required for binding to p110. We also present evidence that short beta hairpins derived from the WW domain retain full p110-binding activity, suggesting a novel route to peptidomimetic development. A cell-penetrating peptide derived from the 20-residue IQ3 motif of IQGAP1 has also shown anti-tumor activity. The IQ3 motif is one of 4 IQ motifs that comprise IQGAP1's IQ domain. We showed that ERK1 and ERK2 bind to the IQ domain, not to the WW domain as was mistakenly believed for many years. We have now found that ERK (and MEK) binding to the IQ domain is both delocalized and cooperative. The delocalized nature of ERK-IQGAP1 binding is shown by the observation that any single IQ domain can be deleted while still maintaining full binding; further, variants containing only two of the four domains (1–2 or 3–4) exhibit >50% binding. Variants containing only a single IQ motif fail to bind, however, suggesting that two or more motifs cooperate to bind ERK. The binding of IQGAP1 to MEK1/2 shows a similar pattern. Collectively, these findings suggest a revised model of IQGAP1-mediated scaffolding, and of how IQGAP1-derived therapeutic peptides might inhibit tumorigenesis.

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Abstract 1319

Structural insights into the formation of repulsive Netrin guidance complexes

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Netrins dictate attractive and repulsive responses during axon growth and cell migration throughout development. The presence of the receptor from the UNC-5 family on target cells results in Netrin-mediated repulsion, while the DCC receptors on target cells can dictate attractive responses. Molecular and structural details of Netrin–UNC-5 interactions, the architecture of Netrin–UNC-5 complexes, and how they signal remain elusive. In this study, we showed that both nematode UNC-5 and UNC-6/Netrin are heparin-binding proteins, determine the structure of UNC-5 bound to a heparin fragment, and validate our structural insights through mutational data. We have engineered the UNC-5–heparin affinity using directed evolution and via structure-based rational design to be used in functional studies *in vivo*. We also showed that UNC-5 and UNC-6/Netrin form a large (>1 MDa), stable and surprisingly rigid complex in the presence of heparin: This complex can incorporate the attractive UNC-40/DCC receptor only at a substoichiometric ratio, demonstrating proposed binary and ternary ectodomain complexes at preparative scale. Our data indicate that UNC-5, in the presence of heparan sulfate proteoglycans, can partly (but not completely) replace DCC in Netrin complexes, resulting in attractive signaling to be switched to a repulsive response. To demonstrate *in vivo* relevance, we show that *C. elegans* with our engineered heparin-binding deficient UNC-5 cannot establish proper gonad morphology due to abrogated distal tip cell migration, which is known to depend on repulsive UNC-5 signaling in response to Netrin. Our work, therefore, reveals that repulsive Netrin responses are mediated through glycosaminoglycan-regulated large macromolecular complexes. In addition, our results also raise the possibility that GAGs and proteoglycans may play a regulatory role in determining attractive vs repulsive responses in axon guidance and cell migration.

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Abstract 1322

H-NOX is necessary for cell-cycle progression in *Caulobacter crescentus*

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Erik Yukl

Cyclic dimeric (3′→5′) GMP (c-di-GMP) is a bacterial secondary messenger that is responsible for regulating things such as motility/sessility, virulence, and cell-cycle progression. The organism *Caulobacter crescentus* has a dimorphic life cycle defined by flagellated swarmer cells and stalked cells. Previous studies have revealed high intracellular c-di-GMP in stalked cells and low c-di-GMP in swarmer cells. Furthermore, it has been shown that deficiency in c-di-GMP production leads to a delayed growth within *C. crescentus*. H-NOX proteins are a family of proteins that regulate c-di-GMP pools by directly interacting with a diguanylate cyclase/phosphodiesterase or a histidine kinase in response to external stimuli such as nitric oxide or oxidative stress. Studies of H-NOX homologues from several facultative anaerobes have demonstrated an influence on biofilm related phenotypes. We thus intended to investigate the effects of knocking out hnox in *Caulobacter crescentus* on biofilm formation, cell growth, and c-di-GMP pools. To address this, we use UV-vis to study bacterial growth over time, crystal violet and fluorescent staining to study biofilm formation, differential expression analyses, and LC/MS/MS to measure cellular c-di-GMP pools. A Δhnox strain of *Caulobacter crescentus* has thus far demonstrated a severe decrease in growth when compared to the wild-type organism grown in a falcon tube and well-plate. In addition, microscopy and crystal violet staining have both indicated the knockout strain demonstrates a biofilm deficient phenotype. Differential expression analyses of these two strains grown in the nutrient rich PYE media has shown a handful of genes related to cell-cycle progression and stalk formation being differentially expressed between these strains indicating a dependence on H-NOX in regulating progression of the cell cycle. Tandem LCMS has also shown significant changes to intracellular c-di-GMP pools. This data indicates H-NOX in the obligate aerobe *Caulobacter crescentus* is used to mediate cell-cycle progression through modulation of intracellular c-di-GMP. To our knowledge this is the first study to correlate H-NOX to the progression of a bacterial cell-cycle.

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Abstract 1334

Designed sensors reveals normal and oncogenic Ras signaling in endomembranes and condensates

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While Ras is known to dynamically shuttle around the cell, the activity, mechanism of activation, and function of non-plasma membrane-localized Ras is unclear due to lack of suitable tools. To address these long-standing questions, we used the Latching Orthogonal Cage-Key pRotein (LOCKR) switch platform to generate first-in-class intracellular sensors for endogenous Ras activity (Ras-LOCKRs) and signaling-dependent proximity labelers (Ras-LOCKRpl). We find that: 1) Receptor activation leads to endogenous Ras signaling at endomembranes, which is enhanced by golgi-localized guanine exchange factors. 2) Recruitment of SAM68 and MARCKS to oncogenic condensates fuels local Ras signaling and cell growth. 3) Major Vault Protein drives RasG12C inhibitor resistance by enhancing signaling at the golgi and altering mitochondrial metabolism. Together, these results highlight the importance of non-plasma membrane Ras signaling (endomembranes and condensates), and our new sensors should accelerate the discovery of new therapeutic targets.

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Abstract 1338

Investigating Drug Fragments, Substrate Mimics, and Metal Ions as Inhibitors and Regulators of the Oncogene Phosphatase Of Regenerating Liver 3 (PRL-3)

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Isabel Snyder, Peter Spielmann, Jessica Blackburn

Phosphatase of Regenerating Liver 3 (PRL-3) has been implicated in the process of cancer metastasis for over two decades. The PRL family consists of a highly conserved trio of prenylated dual-specificity phosphatases sharing roughly 80% identity amongst themselves. One member of this family, PRL-3, has been frequently implicated in cancer metastasis and poor patient prognosis in numerous cancers of epithelial origin. Recent research has shown that the active site of PRL-3 binds the cystathionine beta-synthase (CBS) domain of the CNNM magnesium transport proteins to inhibit magnesium efflux and promote intracellular magnesium accumulation. Disruption of the PRL:CNNM complex blocked key metastatic properties of PRL-3 demonstrating that CNNM binding is key to PRL-3 oncogenic activity. Despite the importance of this discovery, regulation of PRL:CNNM binding is not well understood and there are no therapeutic options to disrupt this interaction. The PRL:CNNM interaction has been shown to modulate intracellular magnesium levels, leading to the hypothesis that this interaction may have some form of cation-sensing mechanism. To test this hypothesis, we investigated the phosphatase activity of PRL-3 against a generic substrate in the presence of calcium, magnesium, or zinc salts to determine how these metals may impact the enzymatic activity of PRL-3. We found magnesium and calcium enhanced phosphatase activity after 30 minutes by 2.2-fold, while zinc concentrations as low as 0.5 mM completely inhibited the reaction, $p = 0.0024$. We hypothesize there is a metal cofactor binding site on PRL-3 that can enhance the phosphatase activity of the protein when magnesium/calcium is bound, but is locked in a rigid conformation upon zinc binding. This metal-sensitive enzymatic activity may therefore serve as a method of modulating the PRL:CNMM interaction within the cell by speeding up the slow release of the inorganic phosphate and allowing more protein to be available for CNNM interaction. Additionally, to identify new inhibitors of PRL-3, we performed a drug fragment screen as well as designed and tested substrate mimetic peptides as potential inhibitor leads. Our drug fragment library yielded several hits that were found to alter the thermal stability of PRL-3, suggesting that these fragments bind the protein and may impact phosphatase activity. We identified six compounds that could disrupt PRL-3 phosphatase activity at 400uM concentrations and further analysis showed two of these compounds induce a 3.6-fold decrease in activity at 30–40uM concentrations when compared to DMSO control treatment, $p = <0.0001$. We also investigated the extent to which peptides mimicking the CBS domain substrate of PRL-3 could bind and block PRL-3's enzymatic

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activity. We designed three cyclic peptides held together by various chemical modifications to mimic the secondary structure of the CBS domains of the CNNM family. Two of our three designs were able to reduce PRL-3's phosphatase activity with the most efficient design showing a 4.2-fold decrease in activity at a 50uM concentration when compared to the DMSO control group, p = <0.0001. We are now working to further develop our PRL-3 fragment and substrate mimic inhibitors as well as characterize how the cation sensing capabilities of PRL-3 might impact CNNM binding. Our long-term goal is to understand how this protein is functioning within the cell with the ultimately inhibit this process in order to disrupt the metastatic cascade.

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Abstract 1350

Investigating the Role of Acetylcholinesterase and Brain-Derived Neurotrophic Factor in Regulating the Levels of Soluble Amyloid Precursor Protein α (sAPPα) in Lung Cancer Cell Media

Hind Al khashali, *Eastern Michigan University*

Ravel Ray, Kai-ling Coleman, Sarah Atali, Ben Haddad, Jadziah Wareham, Jeffrey Guthrie, Deborah Heyl-Clegg, Hedeel Evans

Using two human non-small cell lung carcinoma (NSCLC) cells, we show lower levels of acetylcholinesterase (AChE) and intact amyloid-β40/42 (Aβ), and higher levels of mature brain-derived neurotrophic factor (mBDNF) in the media of H1299 cells as compared to A549 cell media. We also found that the levels of soluble amyloid precursor protein α (sAPPα) are regulated by AChE and mBDNF in A549 and H1299 cell media. The levels of sAPPα were found to be higher in the media of H1299 cells. Knockdown of AChE led to enhanced sAPPα and mBDNF levels and correlated with reduced intact Aβ40/42 levels in A549 cell media. AChE and mBDNF had opposite effects on the levels of Aβ and sAPPα. Treatment with AChE decreased sAPPα levels and simultaneously increased the levels of intact Aβ40/42 suggesting a role of the protein in shifting APP processing away from the non-amyloidogenic pathway and toward the amyloidogenic pathway, whereas treatment with mBDNF led to opposite effects on those levels. Our results are consistent with previous reports demonstrating reciprocal regulation of APP processing into sAPPα and Aβ in that increased secretion of sAPPα is associated with decreased Aβ generation.

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Abstract 1362

Investigating the Signaling Cascades Important in Regulating the Levels of Soluble Amyloid Precursor Protein α (sAPPα) in Lung Cancer Cell Media

Kai-ling Coleman, *Eastern Michigan University*

Hind Al khashali, Ravel Ray, Ben Haddad, Jadziah Wareham, Jeffrey Guthrie, Deborah Heyl-Clegg, Hedeel Evans

Amyloid precursor protein (APP) is a type 1 transmembrane glycoprotein that is widely recognized for its involvement in the pathogenesis and progression of Alzheimer's disease (AD) and neuronal homeostasis. While APP and its processing to amyloid beta (Aβ) have been more extensively studied in AD, the protein is also reported to be expressed ubiquitously by neuronal and non-neuronal cells with frequent overexpression in multiple cancers including lung, prostate, colon, breast, glioblastoma, and pancreatic cancer, driving cancer cell proliferation. In comparing two human non-small cell lung carcinoma (NSCLC) cells, we found differences in the levels of soluble amyloid precursor protein α (sAPPα) in the media of A549 and H1299 cells. Previous reports have shown that the levels of sAPPα are regulated by signaling pathways including protein kinase C (PKC), extracellular signal-regulated kinase (ERK)1/2, and phosphoinositide 3-Kinase (PI3K). We examined potential kinases involved in the regulation of sAPPα levels in the media of A549 and H1299 cells and found that the levels of sAPPα are regulated by protein kinase C (PKC), extracellular signal-regulated kinase (ERK)1/2, phosphoinositide 3 Kinase (PI3K), but not by protein kinase A (PKA).

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Abstract 1365

Interplay Between Nicotine, BDNF, and a β-Adrenergic Receptor Blocker in Regulating Cisplatin Resistance in Lung Cancer Cells

Ravel Ray, *Eastern Michigan University*

Ben Haddad, Hind Al khashali, Jadziah Wareham, Kai-ling Coleman, Danyah Alomari, Jeffrey Guthrie, Deborah Heyl-Clegg, Hedeel Evans

Cigarette smoking is known to be a primary risk factor for the development of non-small cell lung cancer (NSCLC). Here, we investigated the effect of nicotine, brain-derived neurotrophic factor (BDNF), and the β-adrenergic receptor blocker, propranolol, on sensitivity of NSCLC cell lines, A549 and H1299, to cisplatin. Our results show increased cell viability and enhanced cisplatin resistance with nicotine and/or BDNF treatment while propranolol treatment led to opposite effects. Cell treatment with epinephrine or nicotine led to EGFR and IGF-1R activation, effects opposite to those found with propranolol. Blocking EGFR and IGF-1R activation enhanced cell sensitivity to cisplatin in both cell lines. PI3K and AKT activities were upregulated by nicotine or BDNF and decreased upon cell treatment with inhibitors against EGFR and IGF-1R. Co-treatment of cells with cisplatin and inhibitors against PI3K or AKT increased apoptosis and cell sensitivity to cisplatin. Our findings shed light on an interplay between nicotine, BDNF, and β-Adrenergic receptor signaling in regulating survival of lung cancer cells and chemoresistance which might expand therapeutic opportunities that target this regulatory network.

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Abstract 1372

Overexpression of SOX18 Promotes Proliferation, Colony Formation, and Migration in Pediatric Sarcomas

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Jack Ojile, Cherubina Rubannelsonkumar, Chloe Barickman, Terry Shackleford

Ewing's sarcoma (ES) and Rhabdomyosarcoma (RMS) are rare cancers commonly found in children that affect the soft tissue, connective tissue, and bones. Treatments for localized ES and RMS with a prognosis for metastatic sarcomas have a 5-year survival rate between 15 and 30%. Current treatments for ES include various chemotherapeutic drug combinations, and while successful for many tumors, tumors that do not respond to therapy or become resistant are more difficult to treat. In previous studies exploring resistance mechanisms to targeted therapies, a transcription factor SRY-related HMG-box18 (SOX18) was found to be overexpressed and resulted to an increase in the gene expression of a number of receptor tyrosine kinases (RTKs) and increased cell survival. This suggests a role for SOX18 in developing resistance in the presence of targeted treatments. SOX18 is a transcription factor is involved in cell proliferation, growth, wound healing and the development of blood vessels. In recent studies, SOX18 has been shown to have increased expression in various cancers such as colorectal cancer, and breast cancer suggesting it contributes to the tumorigenic process. However, the role of SOX18 in pediatric sarcomas had not been studied. In this study, we evaluated the effect of overexpression and knockdown of SOX18 on cell proliferation, colony formation and migration. Our results indicate that SOX18 overexpression increases these tumorigenic properties and that knockdown of SOX18 shows the opposite effect. A role for SOX18 in tumorigenic processes and resistance mechanisms indicate that it may be a promising biomarker as well as a potential therapeutic target for pediatric sarcomas.

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Abstract 1398

Discovering new molecular mechanisms of cell death

Scott Dixon, *Stanford University*

Non-apoptotic cell death is important for human health and disease. The full complement of non-apoptotic processes that can be activated in mammalian cells is unclear. However, over the last decade we have uncovered several novel mechanisms of non-apoptotic cell death, including ferroptosis and an unusual form of palmitoylation-dependent cell death. I will describe our discovery of these cell death mechanisms and discuss how the application of small molecule probes, genetic screening, lipidomic analysis, and biochemical approaches is leading to new insights into the regulation of both processes. Disrupted lipid metabolism is emerging as one important mechanism governing non-apoptotic cell death and I will describe our efforts to isolate specific lipid metabolic enzymes important for the regulation of non-apoptotic cell death. I will also discuss recent progress in understanding how these non-apoptotic cell death mechanisms can be exploited to treat diseases such as cancer.

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Abstract 1399

Investigating the mechanism of anticancer effects of peptides based on insulin-like growth factor binding proteins

Deborah Heyl-Clegg, *Eastern Michigan University*

Bradley Clegg, Jonathan Devos, Robert Muterspaugh, Deanna Price, Jeffrey Guthrie, Hedeel Evans

There are six IGFBPs (Insulin-like Growth Factor Binding Proteins) with highly conserved structures which regulate cell activity in diverse ways, including inhibition of mitogenesis, differentiation, survival, and other IGF-stimulated actions. Some of these proteins also exhibit IGF receptor-independent activity. IGFBP-3, in particular, has been associated with protection against lung cancer and inhibits the growth and survival of non-small cell lung cancer A549 cells. IGFBP-3 and -5 contain a C-terminal hyaluronan (HA) binding motif [B(X7)B] in which B is either R or K and X7 contains no acidic residues and at least one basic amino acid, and this may be linked to the anticancer activity. In order to investigate the cytotoxicity and possible mechanism of action of these proteins, six analogs were synthesized by solid phase peptide synthesis, containing 18 amino acids derived from the consensus C-terminal domains containing these motifs. A mutant IGFBP-3 peptide lacking the HA-binding motif also was synthesized. Liposome dye leakage, ELISA, and MTT and assays were then utilized to examine effects of these peptides on model membranes, HA binding, and cell viability. All peptides displayed mild to moderate membranolytic activity. IGFBP-3,5, and 6, but not the mutant, blocked HA-CD44 signaling and A549 viability to varying degrees. Overall, results demonstrated an anticancer effect by the IGFBP-3 peptide in particular, which correlated more closely with HA-CD44 interactions than with membranolytic activity.

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Abstract 1410

The daily rhythm of amyloid beta peptide in wild-type and transgenic mice

Valeria Buzinova, *University of Kentucky*

Carrie Johnson, Savannah Turton, Sarah Barth, Samantha Padgett, Katharina Kohler, Haleigh Whitlock, Marilyn Duncan, Michael Murphy

The objective of this study is to investigate the relationship of the circadian rhythm and Alzheimer's Disease (AD) in regions outside of the hippocampus in wild-type (WT) and APP × PS1 knock-in (KI) mice by looking at changes in amyloid beta levels at different time points. We chose to utilize an APPxPS1 mutant knock-in line because they express the amyloid precursor protein (APP) at normal levels under the normal pattern of expression. Mice were acclimated to a 12:12 light:dark cycle for two weeks. Mice were then switched to housing in continuous darkness (D:D) for 24–48 hours. Animals were euthanized in groups of 16 at 3 hour intervals starting after the first 24 hours in D:D (N = 128). Each group contained equal numbers of WT males and females, KI males and females. Time at euthanasia is expressed as Zeitgeber Time (ZT) and correlates to the 24-hour clock, with 7 am = ZT0. Immunoassays were done with the olfactory bulb and cerebellum tissues to determine whether amyloid beta levels oscillated at different ZT points throughout the day. There was similar time-dependent change in amyloid beta in both regions examined. Amyloid beta declined to a minimum at ZT4 (early in the light phase) and peaked in the dark phase between ZT13 and ZT16. Despite less amyloid beta in the cerebellum, the time-dependent pattern of amyloid beta levels was essentially the same. Amyloid beta levels display a circadian rhythm in multiple areas of the brain. Further, these data indicate that this biological process also occurs in WT mice. Funding provided by NIH (AG068215).

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Abstract 1421

The F-box protein FBXL16 upregulates IRS1 signaling and promotes cell growth in lung adenocarcinomas with KRAS mutation

Marion Morel, *Wright State University*

Weiwen Long

F-box proteins are essential components of the SCF (SKP1-CUL1-F-box) E3 ubiquitin ligases in that they bind to SKP1 through the F-box motif and bring the substrate to the E3 ligase complex for ubiquitination. FBXL16 is a poorly studied F-box protein, which was shown to be a transcriptional target of E2F1. Unexpectedly, we recently found that FBXL16 upregulates several oncoproteins targeted by SCF-E3 ligases, such as Steroid Receptor Coactivator-3 (SRC-3) and C-MYC, by antagonizing the activity of another F-box protein, FBW7. By data mining, we found that FBXL16 is highly upregulated in many cancers, including lung adenocarcinomas (LUADs), and its upregulation is associated with poor overall survival. LUAD is the most common form of lung cancer, and about 30% of LUADs harbor constitutively active oncogenic KRAS mutants. However, currently no drug is available for effectively treating LUADs expressing KRAS mutants. Hence, there is an unmet need to identify new therapeutic targets. By immunohistological analysis of lung cancer microarray, we demonstrated that FBXL16 protein is highly expressed in LUADs, and particularly in LUADs harboring KRAS mutations. In line with this, by western blot analysis we found that FBXL16 protein is selectively upregulated LUAD cell lines with activating KRAS mutations. Interestingly, the expression of FBXL16 in these cell lines is strongly correlated with insulin receptor substrate-1 (IRS1) protein expression. By cycloheximide treatment, we found that FBXL16 greatly increases IRS-1 protein stability and level, leading to the upregulation of IGF-1 (Insulin)/IRS1/PI3 K Akt signaling. Importantly, the upregulation of IRS1 protein and its signaling by FBXL16 was confirmed in mouse lungs with conditional overexpression of FBXL16 transgene. Also, depletion of FBXL16 by RNA interference inhibits IGF-1-induced lung cancer cell proliferation and migration whereas FBXL16 overexpression promotes anchorage dependent cell growth *in vitro* and cell invasiveness *in vivo*. Taken together, our findings reveal FBXL16 as a potential target for treating LUADs with KRAS activating mutations.

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Abstract 1426

Single Residue Mutation in Protein Kinase C Toggles Between Cancer and Neurodegeneration

Alexander Jones, *UCSD*

Alexandr Kornev, Jody Weng, Gerard Manning, Susan Taylor, Alexandra Newton

Conventional protein kinase C (PKC) isozymes play a pivotal role in tuning the signaling output of cells, with loss-of-function somatic mutations associated with cancer and gain-of-function germline mutations associated with neurodegeneration. PKC with impaired autoinhibition is removed from the cell by quality control mechanisms; however, mutations associated with neurodegeneration evade these mechanisms, allowing aberrantly active PKC to accumulate. Here we examine why a single residue in the C1A domain of PKCβ, Arg42, results in quality control degradation when mutated to His, identified in two cancers, and bypasses down-regulation when mutated to Pro in the neurodegenerative disease spinocerebellar ataxia. Using FRET-based live cell imaging reporters, we determined that mutation of Arg42 to either His or Pro resulted in reduced autoinhibition as indicated by higher basal activity and faster agonist-induced plasma membrane translocation. Arg42 is predicted to form a stabilizing salt bridge with Glu655 in the C-tail, thus unsurprisingly mutation of this Arg to any residue, including Lys, loosened autoinhibitory constraints. Western blot analysis revealed that whereas R42H had reduced stability, the R42P mutant was stable and insensitive to phorbol ester-induced ubiquitination and down-regulation, an effect also observed by deletion of the entire C1A domain. Molecular dynamics (MD) simulations with local spatial pattern (LSP) visualization, which identifies stable communities in the domain, suggested that the Pro (but not His) at position 42 interacts with Gln66 to impair mobility and conformation of one of the ligand-binding loops. This defect is predicted to be repaired by mutation of the Gln to a shortened Asn. Indeed, the double mutant R42P/Q66N restored degradation sensitivity to that of WT enzyme. Our results combine biochemistry, cell-based assays, structural, and computational biology to unveil the effects of disease-associated mutants that can toggle the same residue to produce gain- or loss-of-function effects on PKC by their impact on the conformation of the C1A domain.

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Abstract 1447

Ubiquitin ligase activity inhibits Cdk5 to control axon termination

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Karla Opperman, Jonathan Amezquita, Gabriel Gaglio, Oliver Crawley, Brock Grill

The Cdk5 kinase plays prominent roles in nervous system development, plasticity, behavior and disease. It also has important, non-neuronal functions in cancer, the immune system and insulin secretion. At present, we do not fully understand negative regulatory mechanisms that restrict Cdk5. Here, we use *Caenorhabditis elegans* to show that CDK-5 is inhibited by the RPM-1/FSN-1 ubiquitin ligase complex. This atypical RING ubiquitin ligase is conserved from *C. elegans* through mammals. Our finding originated from unbiased, *in vivo* affinity purification proteomics, which identified CDK-5 as a putative RPM-1 substrate. CRISPR-based, native biochemistry showed that CDK-5 interacts with the RPM-1/FSN-1 ubiquitin ligase complex. A CRISPR engineered RPM-1 substrate 'trap' enriched CDK-5 binding, which was mediated by the FSN-1 substrate recognition module. To test the functional genetic relationship between the RPM-1/FSN-1 ubiquitin ligase complex and CDK-5, we evaluated axon termination in mechanosensory neurons and motor neurons. Our results indicate that RPM-1/FSN-1 ubiquitin ligase activity restricts CDK-5 to control axon termination. Collectively, these proteomic, biochemical and genetic results increase our understanding of mechanisms that restrain Cdk5 in the nervous system.

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Abstract 1456

The PAR4 Variant P322L Directly Impacts Thrombin Generation

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Elizabeth Knauss, Marvin Nieman

Thrombin is an enzyme that plays an important role in blood coagulation. Thrombin is able to cleave the N terminus of protease activated receptor 4 (PAR4) on the surface of platelets, creating a tethered ligand. Extracellular loop 3 (ECL3), which functions as a gatekeeper for the receptor's ligand binding site, swings out and allows the ligand to bind to the revealed pocket. Upon activation, phosphatidylserine on the inner platelet membrane will flip to the outside, creating a surface for prothrombin to be converted to thrombin, developing a positive feedback loop that activates more platelets. Thrombin cleaves fibrinogen into fibrin, creating blood clots near the damage site. Mutations in ECL3 can disrupt PAR4 reactivity and subsequent platelet activation. A single-nucleotide polymorphism (PAR4-P310L) has recently been associated with a lower risk of developing venous thromboembolism (VTE) in a GWAS (genome-wide association studies) meta-analysis. The questions we are answering through this experiment are how thrombin generation affects coagulation in wild type mice in comparison to mice with a homologous mutation (PAR4-P322L), and if platelet-expressing mutant PAR4 have a difference in phosphatidylserine exposure. For this project we are examining four different genotypes of mice: wild type (C57BI6), P/L (one allele has a replacement of Proline with Leucine in ECL 3 of PAR4), L/L (both alleles have a replacement of Proline with Leucine in ECL 3 of PAR4), and KO (mice where the gene that codes for PAR4 is knocked out). Our hypothesis is that we will observe high levels of blood coagulation in wild type mice, low levels of coagulation on KO mice, and medium levels of coagulation in P/L and L/L mice. With reduced PAR4 reactivity, there will be less thrombin generated, causing less blood clots to form. The method used to assess coagulation and thrombin production is the thrombin generation assay (TGA). Through the calibrated automated thrombogram method we can examine the cleavage of the fluorogenic substrate which is used as a marker for thrombin activity in a plasma sample. The results show that thrombin generation gets progressively lower as PAR4 reactivity decreases, with wild type mice generating the most thrombin and PAR4-KO mice the least ($p < 0.01$). Our findings suggest a key role of PAR4 and ECL3 in platelet activation thrombin generation.

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Abstract 1465

Novel Regulatory Roles of Small G Protein Guanine Dissociation Stimulator (smgGDS) in Insulin Secretion from Pancreatic β-Cells

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Carol Williams, Anjan Kowluru

Background/aims: smgGDS has been implicated in the regulatory control of newly synthesized small G protein prenylation signaling modules. Two splice variants of smgGDS with distinct functions have been reported. smgGDS-607 binds unprenylated small G proteins while smgGDS-558, which lacks one of the thirteen armadillo domains, binds prenylated small G proteins. Extant studies have suggested critical roles for small G protein prenylation in the cascade of events leading to glucose-stimulated (physiological) insulin secretion (GSIS). However, putative roles of smgGDS in insulin secretion remain unknown. The overall objective of the current investigations is to determine the expression of smgGDS in pancreatic beta cells, and to assess their roles in insulin secretion facilitated by variety of insulinotropic stimuli.

Methods: Pancreatic islets were isolated from Sprague-Dawley rats by the collagenase digestion method. Human islets were from Prodo Labs (Alisa Viejo, CA). Clonal insulin-secreting beta (INS-1 832/13) cells were cultured for 45 minutes in the presence of basal glucose (2.5 mM), high glucose (20 mM), KCl (60 mM), or forskolin (2.5 μm). smgGDS antibody was from Santa Cruz Biotechnology (Dallas, TX). GAPDH and Lamin B antibodies were from Cell Signaling (Danvers, MA). β-actin antibody was acquired from Sigma Aldrich (St. Louis, MO). Insulin released into the medium from control siRNA (Horizon Discovery, Lafayette, CO or OriGene, Rockville, MD) or smgGDS-siRNA (OriGene, Rockville, MD) transfected INS-1 832/13 cells was quantified by using an ELISA kit (ALPCO; Salem, NH). Membrane and cytosolic fractions were isolated using the MEM-Per Plus kit (Thermo Fischer; Waltham, MA). Abundance of proteins in cell lysates or subcellular fractions was determined by western blotting and quantified by densitometry.

Results: Immunoblotting studies revealed that both splice variants of smgGDS are expressed in human islets, rat islets and INS-1 832/13 cells. Transfection of smgGDS-siRNA resulted in significant knockdown of both smgGDS splice variants in INS-1 832/13 cells. A significant inhibition (−60%) of GSIS was observed in INS-1 832/13 cells following siRNA-mediated depletion of smgGDS. In addition, insulin secretion elicited by a membrane depolarizing concentration of KCl (increased calcium influx) or by forskolin (increased cAMP generation) was inhibited by −49% and −27%, respectively. Subcellular distribution studies revealed no significant alterations in the abundance of smgGDS in the cytosolic and membrane fractions during the 45-minute period of stimulation of INS-1 832/13 cells by stimulatory (insulinotropic) concentration of glucose. Conclusion: We present the first evidence of expression of smgGDS in human islets, rodent islets, and clonal beta cells. We also demonstrate novel regulatory roles of these proteins in insulin secretion derived from glucose metabolic events, including calcium- and cAMP-dependent signaling steps. Putative roles of smgGDS in the regulatory control of G protein prenylation signaling modules, which are requisite for insulin secretion are in progress.

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Abstract 1467

The Role of Lipin1 in Skeletal Muscle of mdx Mice

Abdulrahman Jama, *Wright State University-Main Campus*

Abdullah Alshudukhi, Steve Burke, Andrew Voss

Duchenne Muscular Dystrophy (DMD) is a genetic disorder inherited through X-linked manner affecting 1 in 3500 male births. It is characterized by mutations on the dystrophin gene which leads to the loss of functional dystrophin protein. The Dystrophin protein is part of a complex of proteins that stabilizes the skeletal muscle membranes due to mechanical stress exerted by movements. Lack of dystrophin leads to membrane tear and damage leading to muscle death through necroptosis. Currently there are no effective treatments for the DMD patients. Lipin1 is a phosphatidic acid phosphatase that converts phosphatidic acid (PA) to diacylglycerol (DAG). DAG is an important molecule that participates in phospholipid biosynthesis. Preliminary data from our lab shows that lipin1 expression, both at the protein and mRNA level, is down-regulated in mdx mice, the DMD mice model. Ablating the remaining Lipin1 levels by generating double knockout mice mdx/lipin1-DKO. In addition, we generated mdx:Lipin1Tg/0 transgenic mice. Our lab seeks to understand the role of lipin1 in DMD.

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Abstract 1477

In vitro reconstitution reveals cooperative mechanisms of adapter protein-mediated activation of phospholipase C-gamma1 in T cells

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Activation of T cells is central to the immune system. Engagement of the T cell antigen receptor (TCR) rapidly leads to activation of protein tyrosine kinases and tyrosine phosphorylation of a number of proteins including the transmembrane adapter protein, LAT. Phosphorylation of LAT enables the recruitment of specific proteins to the plasma membrane such as phospholipase C-gamma1 (PLC-g1). This enzyme when activated catalyzes PIP2 cleavage, which generates IP3 and diacylglycerol (DAG) as second messengers. In epithelial cells or fibroblasts, the molecular mechanism of PLC-g1 activation is dependent on ligand binding to the EGF or FGF receptors, which leads to receptor-intrinsic tyrosine kinase activation. This kinase activation leads to the phosphorylation of a regulatory tyrosine residue on PLC-g1 and its enzymatic activity. However, less attention has been paid to a T cell-specific regulatory mechanism of PLC-g1. Upon T cell activation, PLC-g1, when translocated to the plasma membrane at LAT, also interacts with two other adapter proteins, Gads and SLP-76. We have previously characterized the formation of a tetrameric LAT-Gads-SLP-76-PLC-g1 complex by reconstitution *in vitro* and recently have characterized the thermodynamics of the tetrameric protein complex. In this study, we took a reductionist approach and established an *in vitro* reconstitution system to reveal the functional significance of tetramer formation. We tested PLC-g1 activity *in vitro* using liposomes as a membrane surrogate, focusing on both enzymatic function and localization. We demonstrated that PLC-g1 activation is regulated both independently and additively by 1) recruitment of PLC-g1 to the liposomal surface via phosphorylated LAT, 2) by formation of the LAT-Gads-SLP-76-PLC-g1 tetramer, and 3) by tyrosine phosphorylation of PLC-g1. The recently solved molecular structure of PLC-g1 indicates that, in the resting state, several PLC-g1 domains inhibit its enzymatic activity and contact with the plasma membrane. We propose that the multiple cooperative steps that we observed likely lead to conformational alterations in the regulatory domains of PLC-g1, enabling contact with its membrane substrate PIP2, disinhibition of PLC-g1 enzymatic activity, and production of the IP3 and DAG necessary for T cell activation.

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Abstract 1478

Hexabromocyclododecane (HBCD) Increases Production of Pro-inflammatory Cytokines Interleukin 1 beta (IL-1β) and Interleukin 6 (IL-6) in Human Peripheral Blood Mononuclear Cells (PBMCs)

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The environmental contaminant Hexabromocyclododecane (HBCD) is a brominated flame retardant that is used worldwide in a variety of applications including building insulation, furniture upholstery, textiles for motor vehicle interior and others. As a result, HBCD can be found in various ecological environments, wildlife, human breastmilk, and serum. Interleukin 1 beta (IL-1β) and interleukin 6 (IL-6) are both pro-inflammatory cytokines involved in several biological activities. The primary role of IL-1β is the activation of the immune responses while IL-6 plays a role in cell growth regulation, tissue repair, and immune functions. Inappropriately elevated levels of IL-1β and IL-6 are associated with chronic inflammation and the pathologies that result, such as tumor growth, rheumatoid arthritis, Crohn's disease, and multiple sclerosis. Previous studies have shown that HBCD alters the secretion of both IL-1β and IL-6 from human immune cells. However, it is not clear if these changes are due solely to HBCD alteration of the secretory process or whether it has the ability to change the cellular production of IL-1β and IL-6. This study addresses the hypothesis that HBCD can increase both cellular secretion and production (secreted plus intracellular levels) of these cytokines. Peripheral blood mononuclear cells (PBMCs) were exposed to HBCD at concentration of 5, 2.5, 1, 0.5, 0.25, 0.1, and 0.05 μM for 1 h, 6 h, and 24 h and the secreted levels were measured using ELISA and the intracellular level measured by Western blot. The 24 h exposures to HBCD showed increased production of both IL-1β and IL-6 across all concentrations in cells from multiple donors. These results suggest HBCD is stimulating the immune cells to synthesize IL-1β and IL-6 in the absence of infection or injury. Thus, HBCD may stimulate sustained elevation of IL-1β and lead to chronic inflammation and its attendant pathologies.

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Abstract 1489

The regulation of BRAF by its unique carboxyl terminus via Cdc37/Hsp90 chaperone complex

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The RAF family kinases include three isoforms-ARAF, BRAF and CRAF, whose aberrant activation is responsible for a large portion of cancers. Understanding their regulatory mechanism(s) will facilitate the development of effective targeted therapeutics. Here we found that deleting the carboxyl-terminus destabilized protein and elevated the kinase activity of BRAF but not CRAF or ARAF. Aligning their carboxyl-terminal sequences, we identified an extra cdc37/hsp90-binding region in BRAF except the common 14-3-3 binding motif in all RAF isoforms. To determine how the carboxyl-terminus of BRAF regulates its stability and activity, we next carried a series of deletion analyses, which shows the carboxyl-terminal 14-3-3 binding motif is critical for BRAF stability while the cdc37/hsp90-binding region regulates BRAF kinase activity. Since the Cdc37/hsp90 chaperone complex has been shown to facilitate protein kinase folding but also trap protein kinase in inactive status, we speculated that the carboxyl-terminus of BRAF regulates its stability and activity through moderating the association of Cdc37/hsp90 complex. Indeed, deleting the carboxyl-terminal 14-3-3 binding motif dramatically enhanced its association with Cdc37/hsp90 complex, suggesting that this BRAF mutant without 14-3-3 association is highly unstable. However, deleting the carboxyl-terminal Cdc37/hsp90-binding region of BRAF had only a minor effect on Cdc37/hsp90 association, suggesting that BRAF might have multiple Cdc37/hsp90-binding sites. Hence we scanned the whole BRAF sequence, and found that, besides the well-known Cdc37/hsp90-binding site in Glycine-rich loop and the carboxyl-terminal site, there's at least one more site crossing the DFG motif (GDFG). We thought mutation in single Cdc37/hsp90-binding site did not significantly change the affinity of BRAF with Cdc37/shp90 complex, albeit released its kinase activity. In the COSMIC database, there were a significant number of oncogenic BRAF point mutations falling into the CDc37/hsp90-binding regions such as G466A, G469A, F595L, and A762V, which encode constitutively active mutants. To determine how a weakened Cdc37/hsp90 association triggers the kinase activity of BRAF, we measured the dimer affinity of these Cdc37/hsp90-related BRAF mutants and showed that they had elevated dimer affinity, which accounts for their high kinase activity. Consistently, we also found that these mutants were resistant to the first-generation RAF inhibitor (PLX4720) albeit sensitive to RAF dimer breaker (PLX8394). Together, here we have uncovered an unique regulatory mechanism that governs BRAF stability and kinase activity through fine-tuning Cdc37/hsp90 association, which has important implications in targeted cancer therapy against BRAF mutants.

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Abstract 1491

Effect of NILCO Inhibition on MMP2 and VEGFA in Colon Cancer Cells

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OBJECTIVE: This study aims to evaluate the effect of NILCO inhibition on MMP2 and VEGFA, which are known to play important roles in angiogenesis, invasion, tumor growth, metastasis and patient survival in colon cancer cells. METHODS: The human colon cancer cell lines Caco-2 and HT-29 cells were cultured in DMEM/F12 medium supplemented with 10% FBS and a 100-U/mL penicillin–streptomycin solution and were maintained at 37°C with 5% CO2 and 95% air. Caco-2 and HT-29 cells were treated with Notch1 siRNA, leptin siRNA, IL-1β siRNA, control siRNA alone or in combination with Paclitaxel (PTX; 100 nM for HT-29 cells, 130 nM for Caco-2 cells). After 24 hours treatment, the cells were harvested and processed for PCR analysis. RESULTS: MMP2 mRNA expression was significantly decreased in PTX and Notch1 plus PTX groups compared to the control group in Caco-2 cells. However, MMP2 mRNA expression was decreased in the Notch1, Leptin and IL-1β siRNA-treated groups compared to the control group in Caco-2 cells. In addition, VEGFA mRNA expression was decreased in the cells treated with IL-1β siRNA plus PTX compared to the cells treated with IL-1β siRNA alone in Caco-2 cells. Notch1, Leptin and IL-1β siRNA administered groups has decreased MMP2 mRNA expression when compared to the control group in the HT-29 cell line. Notch1 and IL-1β siRNA administered groups has decreased VEGFA mRNA levels when compared to the control group in the HT-29 cell line. CONCLUSION: In conclusion, our study provides the first evidence that NILCO is a potential biomarker in colon cancer, and targeting NILCO alone as a monotherapy and in combination with chemotherapy may be a promising approach for colon cancer therapy.

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Abstract 1494

Differential Regulation of Rho Guanine Nucleotide Dissociation Inhibitors in Pancreatic Beta Cells Under Metabolic Stress

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Backgrounds/aims: Accumulating evidence implicates Rho G proteins (e.g., Rac1) in acute (i.e., insulin secretion) and long-term (i.e., metabolic dysfunction) effects of glucose in islet beta cells. Extant studies have demonstrated modulatory roles for the guanine nucleotide exchange factors, GTPase activating proteins, and guanine nucleotide dissociation inhibitors (RhoGDIs) in insulin secretion. However, putative regulatory roles of RhoGDIs in the onset of metabolic dysregulation of islet beta cells remain partially understood. Therefore, we undertook the current study to determine the expression of the three known RhoGDIs (i.e., RhoGDIα, RhoGDIβ and RhoGDIγ) in clonal beta cells and human islets and assess the effects of chronic hyperglycemic (metabolic stress) conditions on the expression and subcellular association of the RhoGDIs in pancreatic beta cells.

Methods: INS-1 832/13 were incubated in the presence of basal glucose (2.5 mM; LG) or high glucose (20 mM; HG; metabolic stress) for 24 hrs. Human islets (Prodo Labs; Alisa Viejo, CA) were incubated for 48 hours in the presence of LG or HG conditions. Antibodies against all three RhoGDIs were from Santa Cruz Biotechnology (Dallas, TX). Antibodies against GAPDH, lamin B, and cleaved (active) caspase-3 were from Cell Signaling (Danvers, MA). β-actin antibody was acquired from Sigma Aldrich (St. Louis, MO) The nuclear and cytosolic fractions were isolated using NE-PER Nuclear and Cytosolic Extraction Reagent kit (Thermo Fischer; Waltham, MA). Abundance of proteins in cell lysates or sub cellular fractions was quantified by western blotting and densitometry.

Results: Immunological evidence suggested that RhoGDIα, RhoGDIβ and RhoGDIγ are expressed in human islets and clonal beta (INS-1 832/13 and MIN6) cells. Expression of RhoGDIβ, not RhoGDIα or RhoGDIγ was significantly increased (∼26%) in INS-1 832/13 cells under the duress of HG conditions. Furthermore, exposure of INS-1 832/13 cells to HG significantly increased (∼40%) caspase-3 activation. Interestingly, a significant increase in the degradation of RhoGDIβ (∼35%) was also noted in INS-1 832/13 cells following exposure to HG. Studies in human islets exposed to HG revealed comparable increases in expression (∼21%) and degradation (∼29%) of RhoGDIβ, without eliciting any discernable effects of these conditions on the expression of RhoGDIα and RhoGDIγ. Studies of subcellular distribution of the three RhoGDIs revealed significant association of only RhoGDIα and RhoGDIβ, not RhoGDIγ, with the nuclear fraction under LG and HG conditions.

Conclusions: Based on these findings we conclude that metabolic stress differentially regulates the expression of

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specific RhoGDIs in pancreatic beta cells in that the expression and degradation of RhoGDIβ are significantly increased under the duress of HG in human islets and clonal beta cells. Further, only RhoGDIα and RhoGDIβ appear to associate with nuclear fraction. Putative modulatory roles of increased caspase-3 as the mediator of RhoGDIβ degradation under the duress of metabolic stress leading to the induction of pro-apoptotic events remain to be verified.

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Abstract 1517

Glutamine controls WWOX function via nitric oxide signaling that directly affects cancer cell stemness

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Gene mutation or deficiency may lead to the functional and behavioral changes in cancer cells. WWOX deficient or dysfunctional (WWOXd) cells become metastatic, migrate individually, and undergo retrograde movement upon facing collectively migrating WWOX functional (WWOXf) cells from a distance. Here, we utilize time-lapse microscopy to functionally image WWOXd cells hiding among WWOXf normal cells or benign cancer cells. Compared to WWOXf cells, WWOXd cells have 1) poor efficacy in calcein retention (indicating reduced survival), 2) significantly reduced intracellular nitric oxide (NO), 3) failure in UV-induced calcium influx, 4) increased efficiency in ERK-dependent stress fiber formation during death, 5) undergoing UV-mediated nuclear explosion, and 6) increased pS14-WWOX expression for stemness. In contrast, WWOXf cells maintain normal NO levels, calcium influx capability, calcein retention efficacy, normal mitochondrial membrane potential, but have reduced levels of pS14-WWOX for stemness, and poor efficacy in stress fiber formation. While glutamine is needed for NO production, glutamine depletion from culture media renders WWOXf to exhibit WWOXd phenotypes, including increased stemness, loss of calcium influx, increased stress fiber formation, and converting UV-induced nuclear bubbling cell death to explosion. Functional pY33-WWOX suppresses cancer cell stemness via binding with stem cell maker proteins such as OCT-4, SSEA-4, CD133, and Nanog. Inhibition of pY33-WWOX by antibody induces normal stem cell generation in mice. Together, glutamine activates WWOX function via NO signaling that directly limits cancer cell stemness *in vitro* and *in vivo*.

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Abstract 1523

Blebbistatin and CI-4AS-1 mitigate epileptic seizure via activation of HYAL-2+ CD3- CD19- Z cell

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Yu-Min Kuo, Nan-Shan Chang

As a risk factor for Alzheimer's disease (AD), activated WW domain-containing oxidoreductase (WWOX) with Tyr33 phosphorylation runs against neurodegeneration. A synthetic peptide Zfra4-10 (Zinc finger-like protein that regulates apoptosis) binds membrane hyaluronidase Hyal-2 to initiate the Hyal- 2/pY33-WWOX/Smad4 signaling to restore hippocampus-dependent memory loss in triple transgenic 3xTg mice for AD, suggesting that pY33-WWOX acts together with signaling partner proteins in supporting normal neuronal physiology. An additional mechanism is that Zfra4-10 activates non-T/non-B spleen HYAL-2+ CD3− CD19− Z lymphocytes, which may restore memory loss in 3xTg mice. By chemical library screening, we isolated two small chemicals, blebbistatin and CI- 4AS-1, to trigger the dissociation of MEK/WWOX complex for activating WWOX- induced apoptosis from mitochondria. Wild type and heterozygous Wwoxmice received three consecutive weekly tail vein injections of blebbistatin and CI-4AS. One month later, animals received a convulsive dose of pentylenetetrazol (PTZ) for acute seizure. Blebbistatin and CI-4AS-1 significantly reduced the extent of seizure in the wild type and heterozygous Wwox mice. Blebbistatin and CI-4AS-1 increased the number of surviving neurons against the damage from PTZ in mice as determined by nissl staining. Notably, when blebbistatin-activated Z cells (2×105 to 1.2×106 per mouse) were transferred to both wild type and heterozygous Wwox mice, these cells protected mice against PTZ-induced seizure in a dose-dependent manner. While wild type mice are better protected by activated Z cells than the heterozygous Wwox mice, our observations suggest a protective role WWOX in suppressing seizure.

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Abstract 1526

Phosphoproteomic insights into molecular pattern signaling of green leaf volatiles

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Green leaf volatiles (GLVs) are volatile organic compounds that are immediately synthesized in response to stresses. GLVs can deter herbivores and pathogens, attract predators of herbivores, and prime other plants nearby against possible threats. Therefore, they have the potential to protect crops and reduce pesticide use. However, the pathway that plants perceive GLVs and their signal transduction which leads to such responses are not fully known. Because they are plant compounds that are released in response to cell damage, GLVs are hypothesized to function like damage-associated molecular patterns (DAMPs). In this study, we compared the effect of cis-3-hexen-1-ol (Z3-HOL) and cis-3-hexen-1-yl acetate (Z3-HAC), two typical GLVs, on cultured tomato cells with systemin, a well-known DAMP. We found that the two GLVs induced distinct pH response profiles in a concentration-dependent manner. The change in the extracellular pH is a typical response to DAMPs. Since phosphorylation is an important post-translational modification in cell signaling, we used phosphoproteomics to identify rapidly modified signaling proteins. This study showed phosphorylation of many proteins that are well-known components of molecular pattern signaling pathways upon both GLVs and systemin treatments. From the result, we summarize that MAPK and calcium signaling are involved in the signal transduction of these two GLVs. From gene ontology analysis, Z3-HOL induced phosphorylation of proteins involved in response to stimulus, including immune response, defense response, and response to stress, like systemin. However, Z3-HAC caused more dephosphorylation of proteins in those categories. Our findings indicate that Z3-HOL induces more similar responses as a DAMP in tomato cells, while Z3-HAC established more unique responses. These reveal a part of GLV sensing pathways in tomatoes which could be used as a starting point to elucidate signaling cascades of GLVs and potentially leads to usage in agriculture.

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Abstract 1527

Hijacking Suppression: Anti-CD40 Converts Regulatory T Cells Into Type I Effectors

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Pancreatic ductal adenocarcinoma (PDA) is an aggressive and heterogeneous cancer that is refractory to current treatments. To address this issue, we employ a mouse PDA-derived tumor clone library where individual clones elicit a spectrum of unique immune cell infiltration profiles. We found that the addition of anti-CD40 to the standard anti-PD1, anti-CTLA4 immunotherapy cocktail results in tumor regression only in tumor clones with high numbers of infiltrating T cells. Our question is: what is the mechanism of action for anti-CD40 that empowers therapeutic responsiveness? Our approach will leverage high multiplex microscopy and quantitative image analyses, enabling us to decipher the complexities of cellular behaviors, interactions, and phenotypes within an intact tumor microenvironment (TME). C57Bl/6 mice were subcutaneously implanted with tumor cells into both flanks. Our imaging techniques include: (1) IBEX: iterative immunofluorescence staining method developed in the Center for Advanced Tissue Imaging (CAT-I). (2) Histocytometry: quantitative image analysis. (3) RNAscope: enables visualization of RNA within tissue. Results and Conclusion We found that our therapy uniquely alters regulatory T cells (Tregs) in the TME, severely depleting, reprogramming, and restricting the remaining Tregs to the tumor periphery within 48 hours of anti-CD40 administration. In order to determine the fate of these cells, we used tamoxifen-inducible Foxp3 lineage tracing mice to label all Foxp3–expressing cells prior to therapy initiation. Strikingly, we found that many of the lineage marked Tregs no longer expressed Foxp3. These ExTregs now had high levels of Tbet and IFNg, and had evidence of cognate antigen recognition, as assessed via imaging of NFAT1 nuclear translocation in situ. Single and combination treatments in the absence of anti-CD40 failed to induce this ExTreg phenomenon. Blockade of MHC-II, IL-12, or IFNg or tumor implantation into Batf3 KO, IL12p40 KO, or IFNg KO mice also ablated this phenomenon. These data reveal a unique mechanism by which anti-CD40 amplifies the anti-tumor immune compartment through the conversion of immunosuppressive Tregs to an effector population within the tumor microenvironment.

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Abstract 1546

Stress Upregulates Cytokines that Induce Melanoma Progression

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Recent evidence suggests that the tumor microenvironment (TME) can be altered, in ovarian cancers and others, by stress-induced release of catecholamines such as norepinephrine (NE). Since tumor associated macrophages (TAMs) are known to be key players in melanoma tumor growth and metastasis, we hypothesize that in melanoma, stress-induced catecholamine release results in the stimulation of tumor associated macrophages and secretion of pro-inflammatory cytokines that aid in melanoma progression. To that aim, we set up a co-culture system of melanoma cells and macrophages that were exposed to NE to assay secreted cytokines using a cytokine array dot-blot assay. We show that several cytokines, including angiopoeitin-2, interleukin-11, and macrophage-migration inhibitory factor (MIF), known to contribute to human melanoma progression, were differentially expressed in the co-cultured cells exposed to NE. To validate our results, ELISA were performed. We show a significant increase of MIF concentration in the melanoma/macrophage co-culture exposed to NE as compared to the co-culture without NE exposure ($P < .001$). These results are consistent with our dot-blot assays that stress in the TME increases MIF and possibly other pro-inflammatory cytokines that we are currently validating. Our group and others continue to see evidence demonstrating at a molecular level how stress is implicated as having a major role in tumor progression, emphasizing the importance of including stress management to therapeutic regimens in melanoma and other cancers.

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Abstract 1551

GeranylgeranylTransferase Inhibitors selectively inhibit STIM2-dependent Orai function

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Store Operated Calcium Entry (SOCE) is a vital calcium signaling process facilitating a variety of cellular processes including growth, metabolism and motility. Briefly, SOCE occurs when Stromal Interaction Molecule (STIM) proteins activate and binds to plasma membrane (PM) Orai channels upon ER Ca2+ store depletion, facilitating Ca2+ entry. Prior investigations by us and others have shown that cholesterol inhibits Orai1 function. The mevalonate pathway mediates endogenous cholesterol production, however, there are multiple distinct endpoints in this pathway. Our objective is to investigate the potential mechanisms involved in this pathway by altering GGT function to further understand the fundamental differences in STIM isoform-dependent SOCE. To determine if SOCE was dependent on these other directions, we measured SOCE through confocal microscopy while manipulating each pathway. Interestingly, we have observed a surprising link between geranylgeranylation and SOCE. Briefly, Geranylgeranyl Transferase (GGT) serves a critical role prenylating a number of CAAX-containing PM-associated proteins through the transfer of Geranylgeranyl Pyrophosphate (GGPP). With our results, we have observed dose-dependent SOCE inhibition due to GGT inhibitors (GGTI) in a wide range of cell lines. Interestingly, dose dependence was highly variable; in an effort to determine the reason for this variability, we compared STIM1- and STIM2-dependent SOCE. Remarkably, only STIM2-dependent SOCE was GGTI sensitive. Since STIM proteins do not contain CAAX sites, we are still currently investigating these potential mechanisms to provide conceptual insight into apparent fundamental differences in STIM isoform-dependent SOCE. In addition to potential mechanistic insight, the ability to selectively inhibit the STIM isoforms could have significant therapeutic potential by providing a degree of cell selectivity for control of SOCE, a universal process.

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Abstract 1552

Acetyl Tributyl Citrate (ATBC): A Non-phthalate Plasticizers' Senescence Evoking Effects in Neuro2a cells

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Lucinda Carnell, April Binder

Phthalate plasticizers are noncovalently bound chemicals widely used to impart flexibility into plastics in a variety of household products such as plastic food wrap, personal care products, toys, and medical tubing. Phthalates have been shown to leach out of these plastic products and compromise organismal health by disrupting the nervous and reproductive systems. As a result of these detrimental effects, non-phthalates plasticizers have been developed and are being used as alternatives, but the impacts of phthalate-free plasticizers on organisms has not been extensively tested. Thus, our goal was to examine the effects of the alternative plasticizer acetyl tributyl citrate (ATBC), commonly found in medical devices, cosmetics, and children's toys, on neurons using the mouse neuroblastoma cell line, Neuro2a cells. Previous studies in our lab determined that concentrations ranging from 10 to 200 μM of the phthalate-free plasticizer acetyl tributyl citrate (ATBC) resulted in a decreased number of Neuro2a cells after three days. However, this decrease was not due to cell death as measured by propidium iodide staining. To further examine the mechanism for the decrease in cell number in ATBC-treated cells, we continued this study by examining effects on cell cycle arrest and senescence pathways using flow cytometry, quantitative reverse transcriptase PCR (qRT-PCR), and the senescence associated B-galactosidase expression assay (SA-B gal). Using the Dean-Jett-Fox univariate cell cycle model to fit flow cytometry data based on DNA content, it was determined that cells exposed to 100 μM of ATBC had a significant decrease in the percentage of cells in S/G2 phase. Specifically, in the control, there were 52% of cells in S/G2 phase whereas in ATBC-treated only 37% of cells were in the S/G2 phase ($P = .0036895^{**}$; $N = 5$), indicating cell cycle arrest. To decipher whether this arrest could be a result of cellular senescence, we used qRT-PCR to address the role of two pathways involved in the senescence phenotype; cell cycle arrest and oxidative stress. Following treatment for 24 hours at 100 μM ATBC, the relative mRNA levels of p53 and Nrf2—transcription factors of cell cycle arrest and antioxidant stress response, respectively— were obtained. Gene expression was normalized to GAPDH using the Livak Method, and the log fold change was computed. Both genes were found to be upregulated by 2.08 and 2.34 fold, respectively, with p53 having a significant fold increase ($P = .01943$) and Nrf2 trending towards significance ($P = .09947$) ($N = 6$). To assess activation of senescence more directly, one biomarker of senescence, SA-B gal, expression was measured following treatment of ATBC for three days at a concentration of 100 μM. Preliminary data suggests a statistically significant increase in SA-B gal expression and this

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experiment is being replicated to increase sample size. Together, these results suggest that ATBC is causing senescence in dividing Neuro2a cells. This could have profound implications when considering the outcome of increased loss of astrocyte and/or glial cell function via senescence along with increased neuroinflammation in the context of the aging brain and neurodegenerative diseases.

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Abstract 1554

TIP60-mediated regulation of ΔNp63α is associated with cisplatin resistance

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About 5.4 million basal and squamous cell skin cancers are diagnosed each year in the US. The chemotherapeutic drug, Cisplatin is often used to treat squamous cell carcinoma (SCC) patients, but low response rates and disease recurrence is common. ΔNp63α, a member of the p53 family of transcription factors, is overexpressed and considered oncogenic in non-melanoma skin cancer where it regulates cell survival, promotes proliferation and inhibits cell apoptosis. ΔNp63α has also been shown to promote resistance to cisplatin by transcriptionally regulating several DNA damage response (DDR) genes. A previous study from our lab showed that the histone acetyltransferase (HAT) TIP60 promotes SCC proliferation by positively regulating ΔNp63α protein levels in manner dependent on the catalytic activity of TIP60. This finding suggested that TIP60 may contribute to the failure of platinum-based drugs in SCC and led us to hypothesize that TIP60-mediated acetylation of ΔNp63α regulates its stability and transcriptional activity to promote chemoresistance. Silencing endogenous TIP60 led to a decrease in ΔNp63α transcript and protein levels in multiple SCC cell lines, indicating the positive regulation of ΔNp63α by TIP60 is not cell-line specific. Further, TIP60 levels positively correlated with ΔNp63α stability, protein levels and cisplatin resistance. Stable expression of TIP60 or ΔNp63α individually promoted resistance to cisplatin and reduced cell death, whereas loss of ΔNp63α and TIP60 induced G2/M arrest, increased cell-death and sensitized cells to cisplatin. Moreover, pharmacological inhibition of TIP60 reduced acetylation of ΔNp63α and sensitized resistant cells to cisplatin. Finally, we demonstrated that ΔNp63α and TIP60 levels positively correlated with DNA repair capacity and negatively correlated with cisplatin-DNA adduct formation. Silencing of either TIP60 or ΔNp63α enhanced cisplatin-DNA adduct formation and significantly reduced expression of genes involved in DDR. Taken together, our data indicate that TIP60-mediated stabilization of ΔNp63α increases cisplatin resistance and provides critical insights into the mechanisms by which ΔNp63α confers cisplatin resistance through regulation of genes involved in DNA damage repair. Our findings suggest that inhibition of TIP60 may be therapeutically advantageous in overcoming cisplatin resistance in SCC other epithelial cancers.

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Abstract 1562

Investigating molecular mechanism of checkpoint inactivation in budding yeast

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Fangli Weng

DNA damage is one of the most common stresses that can impact cell health. In response to DNA damage, cells can activate a sophisticated DNA damage response (DDR) pathway to preserve genomic stability. Misregulation of DDR can lead to genetic instability and cause diseases such as cancer. Therefore, it is essential to understand the molecular mechanisms of DNA repair and their regulation. In budding yeast *Saccharomyces cerevisiae*, DNA damage response has two phases consisting of initial checkpoint activation for repair and the subsequent checkpoint inactivation for adaptation. Ddc2 is a protein required for the recruitment and activation of checkpoint kinase Mec1 in yeast. Previous studies suggest that Ddc2 degradation is involved in the inactivation of checkpoint but the mechanisms by which Ddc2 is degraded remain unclear. In this study, we find that prolonged methyl methosulfate (MMS) treatment leads to degradation of both endogenous and overexpressed Ddc2. Interestingly, MMS-induced Ddc2 degradation is partially impaired by MG132, a specific inhibitor for proteasome. Likewise, MMS-induced degradation of Ddc2 is also partially blocked in a proteasome-disruptive mutant. Notably, the proteasome-disruptive mutant displays a substantially delayed inactivation of checkpoint. Taken together, these results reveal the involvement of the proteasome ubiquitin pathway in degrading Ddc2 and in checkpoint inactivation.

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Abstract 1568

Comparison of TLR4 and TLR1/2 in Pentachlorophenol Induced Stimulation of IL-1β in Human Immune Cells

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The environmental contaminant pentachlorophenol (PCP) is detected in human blood samples at levels as high as 5 μM. Exposure to PCP presents a significant risk to humans. PCP has been associated with respiratory diseases and cancer, showing a strong association with non-Hodgkin's lymphoma, multiple myeloma, and kidney cancer. Interleukin-1 βeta (IL-1β) is a potent pro-inflammatory cytokine produced by immune cells. Production of IL-1β by immune cells is normally stimulated when pathogen- or damage-associated molecular patterns (PAMPS/DAMPS) activate the toll-like receptor (TLR) regulated pathways. Various TLRs are expressed in immune cells and nonimmune cells. TLRs are categorized based on their localization within the cells as either cell surface or intracellular receptors. TLR4 and TLR1/2 are predominately cell surface receptors. It is well known that abnormal production of IL-1β is responsible for chronic inflammation (inflammation in the absence of injury or infection), which is implicated in several diseases such as autoimmune diseases and cancer. Previous work has demonstrated that PCP causes human immune cells to produce elevated levels of IL-1β and that PCP-induced stimulation of IL-1β production was dependent on the activation of MAP kinases, which are components of TLR signaling pathways. It has not yet been established whether PCP associates with TLR4 and TLR1/2. In this study, we examined whether PCP requires TLR4 and TLR1/2 to stimulate the production (secreted + intracellular levels) of IL-1β in human peripheral blood mononuclear cells (PBMC). Cells were treated for 1 h with a selective TLR4 inhibitor (TAK242) and TLR1/2 inhibitor (CUCPT22) or appropriate control, prior to exposure to 5, 2.5, and 1 μM PCP. Secreted IL-1β was measured by ELISA and intracellular IL-1β was determined by Western blot. The results indicate that PCP-induced stimulation of IL-1β productions was diminished in immune cells where TLR4 was inhibited with TAK242. However, the production of IL-1β was not consistently diminished in immune cells where TLR1/2 was inhibited with CUCPT22. The research findings suggest that the PCP-induced production of IL-1β is dependent upon TLR4 receptors and independent of TLR1/2 receptors in human immune cells. These results offer an insight into the mechanism by which PCP may lead to chronic inflammation and its associated pathologies.

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Abstract 1583

A Novel Oridonin Analog CYD0682 Protects Against Liver Fibrosis through Inhibiting HSP90/NF-κB Pathway in Hepatic Stellate Cells

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Introduction: Liver fibrosis is a progressive, pathologic process that may result in cirrhosis, portal hypertension, liver failure and hepatocellular carcinoma. Activation of hepatic stellate cells (HSC) represents the initiation of liver fibrogenesis since activated HSC are the major producer of extracellular matrix proteins (ECM). Chronic inflammation is an important inducer for liver fibrosis, cirrhosis and potentially liver cancer. NF-κB was identified as a key inflammatory pathway as well as a modulator of activated HSC survival. Inflammatory cell derived TGFβ1 activates HSC and is the most known potent agonist of fibrosis. Previously, our team reported that natural medicine compound oridonin and its novel analog CYD0682 suppressed HSC proliferation and ECM production, however, the underlying molecular mechanisms remain largely unknown. Purpose: In this study, we determined the effects of CYD0682 on NF-κB signaling and its associated factor HSP90 on activated HSC *in vitro*.

Methods: Activated human and rat HSC lines LX-2 and HSC-T6 were used. Cells were treated with either CYD0682 or HSP90 inhibitors, then exposed to lipopolysaccharide (LPS) or TGFβ as indicated. Nuclear and cytosolic proteins were isolated for Western blots or immunofluorescence assay.

Results: CYD0682 treatments inhibited LPS-induced IKKα/β phosphorylation, IκBα phosphorylation/ degradation, NF-κB/p65 nuclear translocation and DNA binding activity. LPS-induced NF-κB target proteins ICAM-1, IL-1β and IL-6 were suppressed by CYD0682 in a dose-dependent manner. Endogenous and LPS-induced p65 phosphorylation at Ser536 was inhibited by CYD0682. Notably, CYD0682 treatment dose-dependently down-regulated the expression of IKKα/β, RIP1, AKT, Stat3, FAK and CDK9, all of which are HSP90 interacting proteins, suggesting that HSP90 may be involved in CYD0682 regulated NF-κB signaling and fibrogenesis in HSC. Our results revealed that HSP90 specific inhibitors 17-AAG and CCT018159 prevented LPS-induced IκBα phosphorylation and degradation, p65 nuclear translocation and DNA binding. Similar to the effects of CYD0682, HSP90 inhibitors suppressed endogenous and TGFβ-stimulated fibrosis markers collagen type I and fibronectin.

Conclusion: The anti-fibrogenetic effect of novel oridonin analog CYD0682 is through the HSP90 regulated canonical NF-κB pathway. *In-vitro*, CYD0682 demonstrates promise as an antifibrosis treatment.

Work is supported in part by NIH grant T32 GM008256-30.

Abstract 1604

Elucidating the anticancer mechanism of an antipsychotic agent for the treatment of Glioblastoma

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Glioblastoma (GBM) treatment has a myriad of challenges, such as the impermeability of chemotherapeutics through the blood-brain barrier (BBB), drug resistance to standard care therapy such as temozolomide (TMZ), including severe toxicity due to high doses necessary for the action. With the current therapies proving inadequate to elicit the desired response, we aimed at rechanneling an FDA-approved antipsychotic agent, Pimavanserin tartrate (PVT), for GBM and attempted to elucidate its mechanism of action. The antineoplastic property of PVT was unraveled by performing a Sulfarhodamine-B cytotoxicity assay on human and murine GBM cell lines. PVT inhibited the growth of GBM cells at a 50% inhibitory concentration (IC50) in the range of 5–8 μM, whereas the IC50 of TMZ was around 1000 μM, 200 times higher than PVT. PVT also enhanced the efficacy of TMZ by reducing its IC50 when given in combination. The mode of cell death caused due to PVT was found to be apoptosis. This finding was concluded based on immunoblotting of several pro-apoptotic proteins, such as cleaved caspase-3 and Bim, which were upregulated due to PVT. Moreover, the annexin-V assay was performed using flow cytometry to confirm the mode of cell death to be apoptosis. 10 μM PVT treatment of GBM cells lead to a 70% increase in the apoptotic cell population. We further screened several oncoproteins to discover the modulation caused by PVT in GBM cells. It was found by western blotting that PVT downregulated the Akt phosphorylation. Further, it was seen that there was an increase in the expression of FOXO proteins, negative downstream regulators of Akt, which get translocated to the nucleus leading to increased transcription of Bim. Thus, we found that PVT regulates the PI3K/Akt signaling in GBM cells leading to their apoptosis. To corroborate our findings and confirm if the PVT crosses the BBB, we performed an orthotopic *in vivo* study of PVT. Luciferin-transfected GBM cells (CT2A-Luc) were intracranially injected in the bregma of immunocompetent C57BL/6 mice, and PVT (10 mg/kg-the human equivalent dose) was administered orally every day. Tumor growth was monitored by checking the luminescence of the cells using an IVIS imager. We observed a significant decrease in the tumor growth of PVT-treated mice with no organ toxicity. All the pre-clinical investigations involving

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animals were carried out in line with the ethical standards laid out by the Institutional Animal Care and Use Committee (IACUC). Thus, we conclude that PVT suppresses the GBM progression by modulating the PI3K/Akt/Bim signaling axis with minimal toxicity profile.

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Abstract 1609

Investigating Genetic Underpinnings of Depression

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Current estimates of worldwide depression incidence are over 280 million people, with women and those over 50 at even greater risk. Research has found numerous potential genetic links, including those within the hypothalamic-pituitary-adrenal (HPA) axis, specifically with relation to glucocorticoids, and within the immune system, particularly as it relates to cytokine production and function. Based on this, we have hypothesized that single nucleotide polymorphisms (SNPs) in glucocorticoid receptors (GR) and corticotropin releasing hormone receptor 1 (CRHR1), as well as interleukin 1 (IL-1B) and cyclooxygenase 2 (COX-2) may predict incidence of depression. Our study investigated the genotypic frequency of several SNPs related to both HPA axis and immune system function. Buccal swabs provided DNA and were acquired from both patients being treated from psychiatric illness, and from a control population. Extracted DNA was analyzed using a combination of allele-specific polymerase chain reaction (asPCR), as well as quantitative polymerase chain reaction (qPCR) to determine allelic frequency associated with each SNP. Participants also completed the Center for Epidemiologic Studies Depression Scale (CES-D) and the Mini Mood and Anxiety Symptom Questionnaire (Mini-MASQ). Participant scores on these instruments included a range of scores, encompassing absence of depression, mild depressive symptoms, and severe depressive symptoms. Preliminary data from asPCR of rs2070951 did not show correlation between depressive symptoms and genotype. Future analysis is predicted to uncover links between other SNPs and depressive scores. Understanding the connection between depression and genotypic variation can help shape diagnosis and treatment in the future, with the goal of improving patient outcomes.

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Abstract 1610

Elucidating the Role of Site-Specific Lysine Acetylation in Thyroid Hormone Receptor β1 Intracellular Localization

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Thyroid hormone receptors (TR) are nuclear receptors that regulate key physiological and developmental processes in a thyroid hormone-dependent manner. Although primarily nuclear localized, TRs rapidly shuttle between the nucleus and cytoplasm through nuclear pore complexes via nuclear localization signals (NLS) and nuclear export signals (NES). Prior studies in our lab identified specific importins and exportins that mediate shuttling of two subtypes of TR, TRβ1 and TRβ1. Intracellular localization can also be influenced by post-translational modifications of TRs. A prior study in our lab revealed that the acetylation of three lysines (K184, K188, and K190) within the NLS of TRβ1 produced a significant cytosolic shift in comparison to wild-type TR. To determine whether acetylation of all three sites is required for altered localization, we transfected HeLa cells with GFP-tagged TRβ1 single or triple site acetylation mimics (K184Q, K188Q, K190Q), GFP-tagged TRβ1 single or triple site non-acetylation mimics (K184R, K188R, K190R), and wild-type TRβ1. Fluorescence-based nucleocytoplasmic ratio (N/C) scoring revealed a significant decrease of the N/C in each single site acetylation mimic, relative to wild-type. Average relative N/C's of the single site acetylation mimics were not significantly different from the triple site (Q) acetylation mimic (Q = 0.65; K184Q = 0.79, $p = 0.19$; K188Q = 0.62, $p = 0.59$; K190Q = 0.67, $p = 0.68$), indicating that the acetylation of one site alters intracellular localization to the same extent as the acetylation of all three sites. The average relative N/C's of the single site non-acetylation mimics also were not significantly different from the triple site non-acetylation mimic or from wild-type TR ($p > 0.05$), suggesting that the inability to acetylate either a single site or all three sites of TR does not impact intracellular localization. Overall, these findings further characterize the role of acetylation as a regulatory switch for TR intracellular localization.

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Abstract 1614

Genetic Circuits to Promote Cancer Rejection via Tumor Expression of Alloantigens

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Background: Cancer immunotherapy harnesses the immune system to eradicate cancerous cells. However, reducing systemic toxicity in patients remains a clinical unmet need. To improve immunotherapies' safety, my supervisor previously developed genetic circuits that "hijack" and force tumor cells to release therapeutics promoting immune responses against the tumor itself, without harming healthy tissues.

Objective: This project's objective was to develop a genetic circuit expressing allogeneic antigens (alloantigens), a novel immunotherapeutic output, to promote tumor allorejection in mouse strains. Alloantigens are antigens exclusively native to certain individuals and immunologically "foreign" to other individuals of a species. We proposed that if the genetic circuit "hijacked" tumors of the C57BL/6 mouse strain to express MHC Class I alloantigens derived from a different strain (BALB/c), the C57BL/6 tumors alone would become "foreign" to the body, leading to tumor organ rejection via CD8+ T-cell mediated allorejection.

Methods: The circuit comprises 3 modules: Modules 1 and 2 are TF-sensor modules, which harness tumor-specific activity of transcription factors (TFs) and promoters (sensors); driven by the TF-sensor modules, Module 3 is the output module that enables tumor cells to secrete any genetically encodable output (e.g., alloantigens). To validate TF-sensor modules' tumor specificity, cancerous (OVCAR-8) and healthy (IOSE) cells were lentivirally infected with synthetic sensors associated with cancerous TFs. Flow cytometry elucidated the ratio of sensors' fluorescent intensities between both cell types. The most tumor-specific sensors were selected for TF-sensor modules to drive output module expression. To construct the output module, BALB/c alloantigen sequences were amplified from murine spleen mRNA. To assemble the complete 3-module circuit, cancerous cell lines, BPPNM and YUMM1.7, were lentivirally infected with the TF-sensor modules and, upon recovery from infection, were re-infected with the output module of BALB/c mouse alloantigens. These cell lines were injected into C57BL/6 mice for *in vivo* validation.

Results: BPPNM models: Tumor burdens for each mouse group were measured with *in vivo* bioluminescent imaging. The mouse group injected with the alloantigen-genetic circuit had a tumor burden that was 51.1% less than the negative control tumors (for which no therapeutic was added) 5 days post-tumor inoculation, indicating that the alloantigens may have curtailed *in vivo* tumor growth. YUMM1.7 models: For mice injected with the alloantigen-genetic circuit, tumors grew more slowly than the negative control, persisting for 19 days post-tumor inoculation. In the negative control mice, tumors grew

aggressively and ulcerated in the mouse, prompting euthanization 10 days post-tumor inoculation.

Conclusion: During this project, we constructed a genetic circuit that "hijacked" tumors to express alloantigens and demonstrated anti-tumor efficacy in mouse models. These preliminary results suggest that intratumoral alloantigen expression may have hindered tumor growth via CD8+ T-cell recruitment. Extensions of this project can include methods for reducing tumor's immunosuppression, such as knocking out genes that hinder the complement immune response. This project serves as a proof-of-concept of the circuit's modularity and specificity, which allows for safer, tumor-localized therapeutic delivery. Thus, the genetic circuit's methodology has potential to address the unsolved issue of systemic toxicity of cancer immunotherapy.

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Abstract 1620

Cellular RNA interacts with MAVS to promote antiviral signaling

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Immune signaling is tightly regulated to promote pathogen clearance while preventing tissue damage. An antiviral innate immune response is activated by the detection of viral RNA motifs by RIG-I-like receptors (RLRs) which induce interferons and antiviral genes. Signal transduction downstream of RLRs proceeds when several effectors coalesce at a multi-protein complex organized around the adaptor protein MAVS, termed the MAVS signalosome. Many protein-protein and protein-membrane interactions as well as post-translational modifications are required for the proper formation and function of the MAVS signalosome. While RNA molecules have been shown to modulate protein complex function by serving as molecular decoys, guides or scaffolds, whether RNA plays an architectural role at the MAVS signalosome remains unknown. We have found that MAVS directly interacts with cellular RNA through its conserved central intrinsically disordered domain. Ribonuclease (RNase) treatment disrupts the migration of MAVS and MAVS signalosome proteins through a sucrose gradient, indicating that RNA promotes MAVS signalosome formation. RNase treatment also inhibits *in vitro* phosphorylation of the transcription factor IRF3 by the MAVS signalosome during immune activation by viral RNA and non-RNA RIG-I agonists. Together, these findings support the hypothesis that cellular RNA molecules promote key protein-protein interactions at the MAVS signalosome for efficient immune activation. Therefore, this work has uncovered new RNA-centric mechanisms by which antiviral immune signaling is regulated.

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Abstract 1636

Progranulin modulates RYK and EGFR activity in mesothelioma cells

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Mesothelioma is an aggressive disease with poor prognosis and limited therapeutic options. A better understating of mesothelioma biology might lead to the identification of novel therapeutic targets. Progranulin is a pleiotropic growth factor often dysregulated in cancer. In many tumor models progranulin has a pro-tumorigenic role, but the molecular mechanisms of progranulin oncogenic action are not fully elucidated. In bladder cancer, progranulin promotes tumor cell migration, invasion, and *in vivo* tumor growth by triggering EphA2 non-canonical signaling. Recently, we have demonstrated a critical role for progranulin in mesothelioma, where it modulates cell migration, invasion, adhesion, and *in vivo* tumor formation. Significantly, we also showed that EphA2 is not the major functional receptor of progranulin in mesothelioma, where it activates alternative RTKs, including EGFR and RYK, a co-receptor of the WNT signaling pathway. In addition, we demonstrated that progranulin modulates focal adhesion turnover, a key process in cell migration and invasion, by modulating FAK phosphorylation in a RYK-dependent manner. Thus, our data suggest that progranulin regulates a complex crosstalk between EGFR, RYK and FAK, but the molecular details of this functional interaction are not yet defined. Using immunofluorescence, we demonstrated progranulin and RYK colocalization in mesothelioma cells. Interestingly, progranulin and RYK co-localization mostly occurs in intracellular vesicles, suggesting a potential role for progranulin in regulating RYK internalization/recycling. This hypothesis is also supported by preliminary data in cycloheximide-treated GRN KO MSTO-211H cells were stimulation with recombinant progranulin determined downregulation of RYK as compared to untreated cells. Similarly, in the same experimental conditions, we also observed a downregulation of EGFR levels upon progranulin stimulation. Interestingly, we also observed RYK/EGFR and RYK/FAK co-localization in immunofluorescence experiments. In agreement, co-immunoprecipitation experiments indicated the presence of protein complexes containing RYK and EGFR and RYK and FAK in mesothelioma cells. All together, these results suggest that progranulin oncogenic mechanisms of action might depend on the ability of progranulin to modulate RTKs internalization/recycling and/or the formation of protein complexes containing multiple active RTKs.

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Abstract 1640

Exploration of Electrophysiological Properties of Immortalized Hypothalamic Kisspeptin Neurons *In Vitro*

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Dr. Chappell's laboratory previously immortalized Kiss-1 expressing cells from the arcuate (ARC) and AVPV hypothalamic regions of a female mouse and has explored steroid-influenced expression patterns of multiple genes. Reproduction in female mammals requires temporal coordination of signals within the hypothalamic-pituitary-gonadal axis, in which both negative and positive feedback loops are integrated into select neuronal centers. Neurons expressing Kisspeptin (Kiss-1) are critical mediators of reproductive patency and pubertal progression. Females possess two distinct Kiss-1 neuronal populations, which respond differently to feedback from circulating sex steroids. Mechanisms underlying how steroid hormones influence gene expression and neuronal activity throughout reproductive cycles are incompletely characterized, due in part to the difficulty of studying these dynamic phenomena *in vivo*. Since estrogen (E2) has previously been shown to modulate neuronal activity *in vivo*, we can use these cell lines to more precisely describe the impact of E2 on cellular function; however, baseline activity properties of these cells must be established first. Here, we conducted an electrophysiological characterization of one of these cell lines. Using whole-cell patch clamp electrophysiology, we have assessed intrinsic electrical properties and excitability. We report measures including whole-cell capacitance and resistance, and current-voltage relationships while holding the cells at a range of voltages. We determine that the cell line displays little intrinsic excitability, suggesting it may correspond to the "silent" arcuate kiss1 neuron type, or that immortalization may have suppressed its electrical function. We have begun pharmacological manipulation based on known *in-vitro* gene expression profiles and qPCR investigations of the cell line to further assess the identity and function of these cells. Future experiments will combine electrophysiology, pharmacology, and gene expression studies to explore pathways through which sex steroids may drive changes in gene expression, neuron activity and circuit activity.

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Abstract 1646

Neurodevelopmental deficits precede Purkinje cell loss in a mouse model of Niemann-Pick Type-C disease

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Purkinje cell (PC) loss occurs at an early age in patients and animal models of Niemann-Pick Type C (NPC), a lysosomal storage disease caused by mutations in the Npc1 or Npc2 genes. Degeneration of PCs occurs early in NPC disease; however little is known about how NPC1 deficiency affects the postnatal development of PCs and other cerebellar neuronal populations. Using the Npc1nmf164 mouse model, which is a model of late onset disease, we found that NPC1 deficiency significantly affected the postnatal development of PC dendrites and synapses as demonstrated by significant changes in the levels of synaptic proteins. Atrophy of developing dendrites in Npc1nmf164 PCs was accompanied by changes in the number of postsynaptic spines that colocalized with VGLUT1 + parallel fibers presynaptic terminals. Furthermore, dendrites and presynaptic terminals from parallel fibers were increasingly engulfed by microglial cells coinciding with the decreasing levels of these VGLUT1+ presynaptic terminals by the end of postnatal development. Additionally, PCs dendrites from Npc1nmf164 mice showed decreased levels of mitochondria when compared to WT mice, suggesting metabolic disruption. The developing dendrites of Npc1nmf164 PCs were significantly deficient in mitochondria and lysosomes. Furthermore, anabolic (mTORC1) and catabolic (TFEB) signaling pathways were not only perturbed but simultaneously activated in NPC1-deficient PCs, suggesting a loss of metabolic balance. We also found that mice with conditional heterozygous deletion of the Phosphatase and Tensin Homolog Deleted on Chromosome 10 gene (Pten-cHet), an inhibitor of mTORC1, showed similar early dendritic alterations in PCs to those found in Npc1-deficient mice. The reduction of these presynaptic terminals from parallel fibers in Npc1nmf164 was not due to the loss of granule cells, since the volume of these cells was not different between WT and Npc1nmf164 mice. Our data suggest that disruption of the lysosomal-metabolic signaling in PCs causes dendritic and synaptic developmental deficits that precede and promote their early degeneration in NPC.

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Abstract 1649

Clonal hematopoiesis in experimentally induced colitis

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Introduction/Background: Clonal hematopoiesis (CH) describes the expansion of individual blood cells in the circulatory system and peripheral, functional tissues, associated with a somatic DNA mutation. The development of such a cell mosaic is a natural aging process and is connected with a higher risk of several diseases like cardiovascular diseases (CVD). Besides changes in the blood cells themselves, also changes in the bone marrow niche might be involved in this process. Rising evidence indicates a general connection between conditions with chronic inflammation and increased selection pressure for the development of CH. This indicates the capability of inflammatory bowel disease (IBD) to alter the bone marrow (BM) microenvironment in a way that promotes CH.

Aim/Method: This study aims (i) to analyze alterations in the bone BM microenvironment with effects on inflammatory processes and (ii) to assess a mutual influence of IBD and CH in common driver genes. To construct a human-like low-level CH engraftment scenario the model of adoptive bone marrow transfer (ABMT) was used. CD45.1 Pepboy mice received via retrobulbar injection 5.5 million isolated BM cells from CD45.2 donors on three consecutive days. Donors exhibit either a knock-out or a characteristic mutation in different genes of interest. For the development of experimental colitis, six weeks after ABMT, the Pepboy mice received 2% dextran sodium sulfate (Dss) via drinking water in three-cycle feeds. CD45.2 chimerism in different tissues was assessed by flow cytometry analysis.

Results: Experimentally-induced colitis promotes myeloid hematopoiesis and results in neutrophilia and monocytosis. The IBD-related inflammatory conditions enhance the clonal expansion of Tp53R270H/+ -mutated myeloid progenitor and differentiated cells in the bone marrow and colonic tissue. IL-6 is a major factor in this pro-inflammatory IBD phenotype. These findings suggest a general connection between chronic inflammation and the development of CH with so far unknown downstream effects.

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Abstract 1650

Dynamic changes and correlation between intracellular Ca2+ concentration of neurons and CGRP concentration in plasma after SCI

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Objective: To study the relationship between intracellular Ca2+ concentration of neurons and CGRP concentration in plasma and the severity and duration of spinal cord injury after SCI, as well as the dynamic changes of post-injury concentration, and analyze the correlation between Ca2+ concentration and CGRP concentration after injury.

Methods: First, according to the principle of free fall injury, the disease models of SCI rats with mild, moderate and severe injury levels and the control group were established using "Feeney" method. Then, at different time points after the model establishment, the intracellular Ca2+ concentration of neurons was determined by fluorescence ion imaging and confocal microscope, and the CGRP concentration in plasma was determined by radio-immuno-assay. Finally, the experimental data were analyzed using statistical methods.

Results: The rapid increase of intracellular Ca2+ concentration in neurons occurred in the early stage after the establishment of SCI model, and reached the peak at 24 h after injury. At the beginning of SCI model establishment, plasma CGRP concentration increased sharply (not related to the degree of injury), then decreased rapidly, and reached the lowest peak 24 h after injury. In the SCI moderate and severe injury models, there was a negative correlation between the intracellular Ca2+ concentration of neurons and the change of CGRP concentration in plasma.

Conclusion: Calcium overload and abnormal CGRP concentration in neurons in the early stage of SCI are most likely related to secondary injury of secondary cascade. Therefore, if the homeostasis of neuronal Ca2+ concentration and plasma CGRP concentration is maintained early after SCI, it may be beneficial to the treatment of SCI patients and improve the prognosis, which may become a potential therapeutic direction of SCI in the future.

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Abstract 1652

Effects of brevilin A for apoptosis in chondrosarcoma cells

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Ki Sung Kang

Brevilin A, a sequiterpene lactone isolated from *Centipeda minima* has been studied for various bioactivities such as anti-allergic, anti-bacterial and anti-oxidant. It has been reported that brevilin A exhibits anti-cancer effects against gastric adenocarcinoma, colon adenocarcinoma and hepatocellular carcinoma. However, anti-tumor efficacies of brevilin A in chondrosarcoma cells have not yet been demonstrated. Chondrosarcoma is the second most common tumor of bone and its treatment is difficult and limited to surgery, chemo and radiotherpy. Therefore, we tried to investigate the inhibitory effect and the underlying mechanisms of brevilin A in chondrosarcoma cells. We carried out cell viability in SW1353 cells, which has been used as the established chondrosarcoma cell line and tried to detect the characteristics of apoptosis by DAPI, annexin V and propidium iodide staining. The molecular mechanism related with apoptosis was performed using western blot analysis. The results showed that brevilin A inhibited cell viability in a dose-dependent manner and induced apoptosis with typically morphological changes. Western blot results corroborated Cleaved caspase-8, cleaved caspase-9, cleaved caspase-3 and Bax were up-regulated by brevilin A dose-dependently, while Bcl-2 expression was down-regulated. Taken together, our findings suggest that brevilin A may give a new insight for the anti-tumor effect against chondrosarcoma cells and further studies are required for way better understanding of the mechanism.

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Abstract 1656

Presynaptic scaffold protein, liprin, regulates glucose stimulated insulin secretion and the spatial organisation of exocytosis in pancreatic β-cells

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AIMS: A key feature of insulin exocytosis is that it is targeted towards specialised regions of the β-cell membrane, where β-cells contact extracellular matrix (ECM) proteins of neighbouring islet capillaries. However, how insulin secretion is targeted towards these regions of cell-capillary contact remains unknown. Accumulating evidence suggests that the β-cell-capillary interface forms a specialised secretory domain that is analogous to the neuronal presynaptic active zone (AZ), where synaptic vesicle exocytosis is spatially confined by a complex of presynaptic scaffold proteins. It is now known that several of these presynaptic scaffold proteins (liprin, ELKS, RIM and piccolo) are also present in β-cells and are enriched at the β-cell-capillary interface; however, whether these scaffold proteins facilitate targeted exocytosis in β-cells, as they do in neurons, is unknown. This research investigates presynaptic-like mechanisms for the spatial regulation of exocytosis in β-cells, particularly, the role of liprin in positioning sites of β-cell insulin exocytosis.

METHODS AND RESULTS: β-cells were isolated from humanely sacrificed C57BL/6 mice (approved by local and national ethics). Using live-cell two-photon microscopy to visualise GFP-tagged liprin in mouse β-cells, we show that liprin assembles in small 'islands' or microdomain structures at the β-cell-capillary interface. Upon glucose stimulation, insulin granules preferentially fuse near (<0.3 μm) liprin structures and are excluded from regions of direct overlap with liprin, suggesting that liprin may be involved in tethering granules to specific membrane sites for fusion. We then optimised a protocol for the knockdown of liprin in β-cells using an adenoviral-mediated shRNA approach, confirmed by western blot (42.1% ± 4.0 knockdown). Knockdown of liprin reduces glucose-stimulated insulin secretion (GSIS) and abolishes insulin granule targeting to the β-cell-ECM interface. Finally, to explore the mechanisms underlying the impaired GSIS, we expressed truncated liprin mutant constructs in β-cells, designed to specifically interfere with binding to other presynaptic scaffold proteins. We show that expression of the N-terminus of liprin alone (ELKS- and RIM- binding domains) is sufficient for normal GSIS, while deletion of the N-terminus significantly reduces GSIS, indicating that liprin may function through interactions with ELKS and RIM in an 'presynaptic-like' complex at the β-cell-capillary interface.

CONCLUSIONS: Together, these data show a functional role of liprin in GSIS and the spatial regulation of insulin exocytosis, and are consistent with presynaptic-like mechanisms for the control of insulin secretion in pancreatic β-cells.

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Abstract 1665

The C-terminus of CK1 competes with substrate to bind the kinase domain

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CK1 enzymes phosphorylate substrates that participate in a variety of signaling pathways, yet the mechanisms by which these kinases are regulated remain elusive. Human and yeast CK1s autophosphorylate their C-terminal non-catalytic tails, and C-terminal truncation or dephosphorylation of these enzymes increases substrate phosphorylation *in vitro*. This has led to a model in which the autophosphorylated C-terminus acts as a pseudosubstrate to inhibit catalytic activity. Here, we have directly evaluated this proposed mechanism *in vitro* and *in vivo* using the CK1 homologues *Schizosaccharomyces pombe* Hhp1 and human CK1e. First, we identified the C-terminal autophosphorylation sites, confirming 6 on Hhp1 and 15 on CK1e. Peptides corresponding to the C-termini interact with the kinase domains only when these sites are phosphorylated. Mutating these sites to non-phosphorylatable residues increases the activity of both enzymes towards their substrates. Interestingly, we found that substrates have a higher affinity for the kinase domain than the autophosphorylated C-termini, and substrates can competitively inhibit tail binding even when autophosphorylated. Mutating the basic residues on the kinase domain that comprise the substrate binding groove prevent both tail binding and substrate phosphorylation, supporting the model that tail and substrate compete for binding to the substrate binding groove. Furthermore, we found that the C-termini contribute to substrate specificity, in combination with the previously described autophosphorylation site at T220, which resides in the catalytic domain. Together, these two conserved mechanisms of autophosphorylation can be integrated into a displacement specificity model to explain how CK1 phosphorylates substrates in different pathways under different conditions.

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Abstract 1676

Threshold wounds rejuvenate skin cells via modulation of cellular and mitochondria activities

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Our previous studies have demonstrated that ultraviolet irradiation and oxidative stress induce skin cell damage with a result of skin aging and skin cancer. We have also observed in *in vitro* studies that those external insults damage skin cells in a dose and time dependent manner. Accumulating data have suggested that for certain insults, especially in vogue microneedle treatment in cosmetics, threshold wound or damage exists. Purportedly, at this particular dose, the cells are damaged, but not severely wounded and may eventually be recovered, revived and even rejuvenated. This may translate a better cosmetics benefit, if it occurs in skin cells. Based on our previous studies and unpublished observations, thus, we hypothesize that external insults or wounds, physically or chemically, at the threshold level, may rejuvenate skin cells via modulation of cellular and molecular machineries. To test this hypothesis, we employed various types of insults, including ultraviolet irradiation to damage DNA, hydrogen peroxide to induce oxidative stress, commercially available ACCUTASETM or trypsin to break down cellular matrix, and cosmetically applied microneedles to physically or mechanically damage cells. First, we have determined the threshold wound dose of ultraviolet radiation, H2O2, ACCUTASETM, through the observation of cell viability post treatment. Second, we studied the biochemical, cellular and molecular changes after treatment. Our preliminary data showed that at the dose of 15mJ/cm2, ultraviolet irradiation induces mTOR activation, measured by phosphorylation of ribosomal protein S6. Confocal data showed that microneedle treatment (36 pins) also induces S6 phosphorylation. Interestingly, microneedle treatment appears to affect actin filaments in cytoplasm, reduce mitochondria activity monitored by mitotraker red, using confocal microscopy, and activation of NFκB through p65 translocation in cultured skin keratinocytes. Ongoing studies attempt to focus on the transcription factors associated with cellular rejuvenation, such as those Yamanaka factors (Oct3/4, Sox2, Klf4, c-Myc).

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Abstract 1692

Lysophosphatidylcholine-Docosahexaenoic acid (LPC-DHA), a novel nutraceutical for the prevention of blindness associated with Alzheimer's Disease

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Sugasini Dhavamani

Docosahexaenoic acid (DHA) played a vital role in retinal function. Of all the tissues in the body, retina contains highest concentration of omega-3 fatty acid (FA), docosahexaenoic acid (DHA). Up to 60% of the total FA in rod outer segment membrane phospholipids is DHA. Retina contains dipolyenoic phospholipids with very long chain omega-3 polyunsaturated FA (VLCFA). It is almost completely dependent upon dietary supply of DHA since it cannot synthesize DHA from the linoleic acid (18:3, n-3) precursor. Several epidemiologic and pre-clinical studies shown that dietary omega-3 FA protect against retinal diseases, whereas DHA deficiency impaired visual function. Retinal DHA is significantly reduced in diabetes, retinitis pigmentosa, age-related macular degeneration, and peroxisome disorders, and this deficiency leads to functional defects, impaired visual development and reduced sensitivity. Increasing the retinal DHA at clinically feasible doses has not been possible until now because of the specificity of blood retinal barrier that is incompatible with the specificity of the intestinal barrier. The current study uses the novel approach of dietary LPC-DHA which overcomes both intestinal and blood retinal barriers and improves retinal function. The current belief regarding brain and retinal DHA is that it cannot be increased through diet in adult mammals. We showed here for the first time that it is possible to increase the retinal DHA by almost 100% in normal adult mouse with a low dose of LPC-DHA. This approach thus provides a novel therapeutic approach for the prevention or mitigation of retinal dysfunction associated with AD and diabetes. The major aim of this study is to develop a novel therapeutic approach for the prevention of visual sensitivity decline by increasing retina DHA levels in 5XFAD mice. We tested the hypothesis that LPC-DHA is superior to the currently available DHA supplements (including fish oil and krill oil) in enriching retina DHA and prevention of retinopathy in mouse models of early onset forms of the Alzheimer disease. These studies showed for the first time that retinal DHA can be efficiently increased by dietary LPC-DHA, but not by TAG-DHA or free DHA in Alzheimer mice model. LPC-DHA, but not TAG DHA increased retinal DHA (+96%). TAG DHA, however, had no effect. LPC DHA also preserves *in vivo* retinal structure and function in 5XFAD mice. Since DHA is known to protective against retinopathy and other eye diseases, this study provides a novel nutraceutical approach for the prevention/treatment of these retina degeneration diseases. We believe these studies will have significant effects on the prevention of visual decline in Alzheimer's disease.

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ASBMB

Abstract 1693

Effect of selective inhibitor of E3 ligase, SMER3 on muscle atrophy: potential implication in targeting cancer associated cachexia

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Cancer associated cachexia is a multifactorial syndrome characterized by ongoing loss of skeletal muscle mass with or without the loss of fat mass that cannot be fully reversed by conventional therapy. Cachectic patients have a poor quality of life and decreased efficacy and increased toxicity of chemotherapy. Unfortunately, the therapeutic options for the management of cancer associated cachexia are limited. Therefore, the identification of novel therapeutic avenues to target cachexia is of utmost importance in the management of advanced malignancies. Cachexia is characterized by increased muscle protein breakdown primarily through the activation of Ubiquitin Proteasome Pathway. The muscle specific ubiquitin E3 ligase atrogin-1/MAFbx and Murf1 are among the most studied muscle atrophy genes. Atrogin-1 is specifically induced during cachexia. In this study we evaluated the impact of selective inhibitor of E3 ligase, Skp1-Cullin-F-box (SCF)Met30 ubiquitin ligase, SMER3 in cultured myotubes undergoing dexamethasone induces atrophy. Western blotting analysis showed treatment with SMER3 resulted in decreased atrogin1 expression in dexamethasone treated myotubes. SMER3 was also found to improve the protein levels and reverse the myotube atrophy as measured by the mean myotube diameter of control and treated myotubes. Our study shows that SMER 3 can be used as potential therapeutic against cancer associated cachexia possibly in combination with other standard of care treatments.

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Abstract 1697

Identification of Peptide Sequence Served as Genetically-Encoded or Chemical Probes to Inhibit Gαs in Cells

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Heterotrimeric G proteins (Gαβγ) are molecular switches that alternate between "on" (GTP-bound) and "off" (GDP-bound) states to control the flow of signals from the extracellular milieu to the cytoplasm. As one of four subfamilies of Gα protein (Gαi/o, Gαs, Gαq/11, Gα12/13), Gαs promotes the synthesis of cAMP in the cytosol through stimulation of the enzymatic activity of adenylyl cyclases (AC) after bounds to GTP (active form). Moreover, dysregulation or onco-mutations of Gαs result in diverse human diseases such as cancer and neurodegeneration. Unlike the other three Gα proteins, Gαs lacks reliable tools to specifically inhibit its signaling activity. Herein, we screened peptides from a phage display library and identified two unrelated peptides (GαsBPs) that efficiently bound to active form Gαs (Gαs-GTP) or the constitutively active Gαs R201C mutant, but not to inactive form Gαs (Gαs-GDP). Both peptides present strong binding affinity for Gαs-GTP binding, but barely any binding to Gαs with mutations in its effector binding region. Furthermore, genetically-encoded GαsBPs can block Gs-mediated stimulation of AC both *in vitro* and *in vivo*, and a cell-penetration version can directly pass through the plasma membrane and inhibit Gαs-mediated signaling in cells. In summary, we have identified two peptide inhibitors specific for Gαs-GTP that act by binding to its effector binding site and precluding AC stimulation. These peptides identified here could be leveraged as a drug-like chemical reagent to inhibit Gαs-mediated signaling disorder.

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Abstract 1715

Nonsteroidal anti-inflammatory drug Aspirin downregulates cytokine expression and macrophage activation via NF-κB signalling

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Aspirin (acetylsalicylic acid) is a well-known nonsteroidal anti-inflammatory drug (NSAID) widely used for the treatment of inflammation, pain, and fever. Aspirin is also administered as a blood thinner for the remission of cardiovascular disease (CVD). However, long term use of Aspirin for chronic inflammation and CVD is associated with increased risk of internal bleeding and organ damage. Beyond the roles in COX-inhibition, the detailed mechanism of action and cellular targets of Aspirin remain elusive. Here, we aim to investigate the mechanism of Aspirin mediated anti-inflammatory effects using macrophage as a model system. Our study demonstrates that alongside inhibition of COX2, aspirin down-regulates the expression of various inflammatory cytokines (such as IL-6, IL-1β) and pro-inflammatory genes in macrophages exposed to acute inflammation (LPS-stimulation). Aspirin treatment affects the expression of cytokines both at the mRNA and protein level. Aspirin alleviates the nitric oxide release in LPS-stimulated macrophages, and this is mediated via regulation of iNOS expression. Mechanistic analysis suggests that Aspirin suppresses the inflammation associated with NF-κB. Overall, our studies reveal the mechanism of action of Aspirin-associated anti-inflammatory effects and may help design of novel Aspirin related drugs with higher efficacy and reduced side effects.

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Abstract 1717

Autophosphorylation of tyrosine kinases regulate biofilm formation in the bacteria *Bacillus subtilis*

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Biofilms are multicellular communities of bacteria that form on surfaces, held together by an extracellular matrix of self-produced biomolecules. These colonies can have different properties than other forms of bacteria, including increased antibiotic resistance in healthcare settings. The formation of biofilms is driven by environmental and cellular conditions, many of which are not yet understood. Due to the absence of biofilm activity when they are deleted, two tyrosine kinases, EpsB and PtkA, have been linked to the formation of biofilms in the bacterium *Bacillus subtilis*. However, the biofilm-relevant targets of these kinases, especially PtkA, have not yet been identified. This research combines genetic and protein biochemistry techniques to examine the role of the tyrosine kinases in the regulation of bacterial biofilms. As bacterial tyrosine kinase proteins are known to undergo autophosphorylation at their C-terminus, we examined phosphomimetic mutants of these proteins. We find that autophosphorylation of the proteins affects biofilm formation. Using Western Blot analyses, we examined total phosphorylation of proteins in three separate conditions that produce biofilms. These data show that bacteria forming biofilms in the medium LBGM have the clearest patterns of tyrosine phosphorylation. While more testing is needed in order to validate the results, these findings are a first step in characterizing the biofilm-relevant targets of these tyrosine kinases. An understanding of tyrosine kinases and the regulatory pathways that control biofilm formation can inform how bacteria come together to cause disease, potentially curtailing the infection cycle of various bacterial pathogens.

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Abstract 1732

Disrupting the interaction between mutationally activated Gαq and Gβγ attenuates aberrant signaling

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Heterotrimeric G protein stimulation via G protein-coupled receptors (GPCRs) promotes downstream proliferative signaling. Mutations can occur in Gα proteins which prevent GTP hydrolysis; this allows the G proteins to signal independently of GPCRs and can result in various cancers, such as uveal melanoma (UM). UM is the most common intraocular malignancy in adults. UM metastasizes to the liver in 50% of patients, and there are currently no effective therapies for metastatic UM. Most UM cases harbor Q209L, Q209P, or R183C mutations in Gαq/11 proteins, rendering the proteins constitutively active (CA). The Ras/mitogen-activated protein kinase (MAPK) and Hippo/YAP pathways are commonly stimulated via CA Gαq/11. Although it is generally thought that active, GTP-bound Gα subunits are dissociated from and signal independently of Gβγ, accumulating evidence indicates that some CA Gα mutants, such as Gαq/11, retain binding to Gβγ, and this interaction is necessary for signaling. The main objective of this project is to understand the role of Gβγ in the regulation of CA Gαq/11. We hypothesized that disrupting the interaction between CA Gαq/11 and Gβγ can ultimately inhibit oncogenic signaling in UM. Here, we demonstrate that disrupting the interaction between Gβγ and Gαq is sufficient to inhibit aberrant signaling driven by CA Gαq. Introduction of the I25A point mutation in the N-terminal α helical domain of CA Gαq to inhibit Gβγ binding, overexpression of the G protein Gαo to sequester Gβγ, and siRNA depletion of Gβ subunits inhibited or abolished CA Gαq signaling to the MAPK and YAP pathways. Moreover, in HEK293 cells and in UM cell lines, we show that Gαq-Q209P and Gαq-R183C are more sensitive to loss of Gβγ interaction compared to Gαq-Q209L. Our study challenges the idea that CA Gαq/11 signals independently of Gβγ and demonstrates differential sensitivity between the Gαq-Q209L, Gαq-Q209P, and Gαq-R183C mutants. We propose that disrupting the interaction between CA Gαq/11 and Gβγ can inhibit aberrant cell signaling in UM.

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Abstract 1739

Kinobead competition and correlation analysis (kiCCA) for rapid, sensitive, and high-throughput profiling of kinase interactomes in native cells and tissues

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Protein kinases are enzymes that catalyze reversible phosphorylation, a key process in cellular signal transduction. The 538 kinases in the human protein kinome often integrate with other proteins to form large protein-protein interaction (PPI) networks to fulfill their role as cell signaling hubs in a spatiotemporally coordinated manner. Not surprisingly, the dynamics of these networks are significantly altered in many disease pathways. Kinase-mediated PPI networks are regulated at multiple levels, including protein abundance and post-translational modifications like phosphorylation. Sensitive and high-throughput characterization of kinase PPI networks, i.e., the kinase interactome, can provide critical insight into cellular processes and help elucidate elusive disease mechanisms that may reveal novel drug targets and biomarkers. Commonly used methods to study PPIs typically require the exogeneous expression of epitope-tagged protein in cell lines of interest, which make it difficult to simultaneously monitor dynamic changes in PPIs over different cell lines and signaling states. To overcome these limitations, we developed kinobead competition and correlation analysis, (kiCCA), a mass spectrometry (MS)-based chemoproteomics approach for rapid and multiplexed profiling of endogenous kinase-mediated PPI networks in native cell and tissue lysates. kiCCA uses a panel of 21 multi-targeted kinase probes to compete kinases and their interaction partners from immobilized kinase inhibitor beads (kinobeads), identifying kinases and their PPIs by their similarity in MS signals across the 21 kinobead pulldown experiments. We used kiCCA to profile the kinase interactomes of 18 diverse cancer cell lines and to study dynamic changes in kinase PPIs in an acute signaling model of epidermal growth factor-stimulated HeLa cells. We identified and quantified 1,154 high-confidence PPIs (Pearson's r-value $\geq$ 0.6) between 238 kinases and684 non-kinase proteins and showed that kinase PPI networks are highly dynamic and context dependent. We found that the PPI network dynamics of the AP2 Associated Kinase 1 (AAK1) was among the most frequently altered between different states in cell lines and clinical samples of liver cancer. We validated the AAK1 complex with co-immunoprecipitation MS and showed that RNAi knockdown of AAK1 and its non-kinase protein interactors in liver cancer cell lines led to the upregulation of cell cycle-related kinases and increased sensitivity (up to 18-fold shift in EC50) to cell cycle checkpoint kinase inhibitor drugs. In summary, kiCCA is a powerful approach to study dynamic kinase interactomes and their roles in signaling pathways in native cell and tissues.

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Abstract 1746

Tolerization of Toll-like Receptor 4 using Engineered Lipid A Mimetics Inhibits Ultraviolet Radiation-Induced Apoptosis

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Ultraviolet (UV) radiation induces DNA damage resulting in epidermal cell mutations and immunosuppression which can contribute to the formation of nonmelanoma skin cancer. UV-induced cellular damage is commonly associated with apoptosis characterized by a non-inflammatory cell death through the cleavage and activation of caspase-3. Toll-like receptor 4 (TLR4) is a cell associated innate immune receptor and has been shown to contribute to UV-induced carcinogenesis. UV-induced apoptosis requires TLR4 signaling to activate the apoptotic signaling cascade, if TLR4 is absent then UVB exposure causes cells to die by a more inflammatory process called necroptosis. We hypothesize that skewing cell death from apoptosis to necroptosis using lipid A mimetics to reduce TLR4 expression through tolerization can reduce apoptosis and lead to inflammatory cell death. Hexa-acylated and penta-acylated lipid A memetics from *Escherichia coli* (*E. coli*) are used to tolerize TLR4 in bone marrow-derived macrophage (BMDM) cell lines. This will likely lead to the BMDM cells being less susceptible to UV-induced carcinogenesis. To analyze the role of TLR4 in UV-induced carcinogenesis, cells that are TLR4-proficient, TLR4-deficient, and TLR4 tolerized with hexa-acylated or penta-acylated lipid A were exposed to multiple doses of UVB radiation. UV-irradiated BMDM cells show cleavage of the DNA, creating a ladder whereas UV-irradiated BMDM cells incubated with lipid A show a lack of DNA cleavage. UV-irradiated BMDM cells were deficient in DNA repair as opposed to those treated with lipid A. The data suggests that tolerization of TLR4 using canonical ligands may be a useful tool in promoting DNA repair and maintaining immune responses following UV-induced damage. Our data indicates that exposure to lipid A attenuates TLR4-mediated apoptotic cell death and, likely, downstream immune suppression. TLR4 ligands may be useful in prophylactic treatment approaches against UV-induced carcinogenesis.

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Abstract 1750

The *C. elegans* PVD dendrite branching receptor HPO-30 regulates actin polymerization through two distinct mechanisms

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The formation of dendritic branches is critical for the formation of complex neural circuits, but the exact molecular mechanisms that drive dendrite branching remain largely unknown. Previous studies using the *C. elegans* PVD neuron as a model system identified a group of proteins, which form a co-receptor complex to transmit extracellular guidance cues to intracellular actin remodeling machineries. In this complex, the transmembrane protein HPO-30 directly uses its intracellular domain (ICD) to recruit a central actin nucleation promotion factor, the WAVE regulatory complex (WRC), to the membrane, where the WRC can stimulate the Arp2/3 complex to polymerize actin. Here, by combining biochemical and structural analysis, we show that HPO-30 ICD is intrinsically disordered, but requires dimerization to bind the WRC efficiently. The interaction subsequently promotes WRC activation by the GTPase Rac1. In parallel, the dimerized HPO-30 ICD can also directly bind to the sides and barbed ends of actin filaments. Binding to the barbed end prevents both actin polymerization and depolymerization, similar to the capping protein CapZ. Our study provides an intriguing model that shows how membrane proteins can fine-tune actin remodeling through distinct mechanisms to promote neuron morphogenesis.

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Abstract 1752

The Effect of Resistance to Thyroid Hormone Mutation C392X on the Nuclear Export of Thyroid Hormone Receptor Alpha 1 via Exportin 5 and Exportin 7

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Vincent Roggero, Lizabeth Allison

Thyroid hormone receptors (TRs) typically shuttle between the nucleus and cytoplasm. Within the nucleus, they can influence gene expression in a manner dependent on thyroid hormone binding. Resistance to Thyroid Hormone (RTH) patients produce thyroid hormone, but their TRs do not appropriately respond, preventing proper metabolism and development. We studied the activity of a TR mutant that can lead to RTH, the C392X mutant of TRα1, which lacks one of its three nuclear export signals (NES). Specifically, we investigated the effect of exportin 5 (XPO5) and exportin 7 (XPO7) on the nuclear localization of this mutant, hypothesizing that the wild-type TRα1 would be more cytoplasmic in the presence of these exportins, while the localization of the C392X mutant lacking an NES would be unaffected. We co-transfected HeLa cells with either wild-type mCherry-TRα1 or C392X mCherry-TRα1 and either GFP-XPO5, GFP-XPO7, or GFP alone. After fixing the cells, we imaged them by fluorescence microscopy, and then determined the fluorescence intensity of mCherry-TRα1 within representative areas of the nucleus and cytoplasm of each cell to produce a nuclear-to-cytoplasmic ratio (N/C). There was no significant difference in the C392X mCherry-TRα1 N/C upon the addition of GFP-XPO5 compared to C392X mCherry-TRα1 co-transfected with GFP alone, though the wild-type N/C became significantly more nuclear when co-transfected with GFP-XPO5 compared to GFP alone ($p = 0.038$). There was no statistical difference in localization of mutant or wild-type TRα1 when co-transfected with GFP-XPO7 compared to co-transfection with GFP alone. Results suggest that the C392X mutation may alter the interaction of TRα1 with XPO5 but does not affect interactions with XPO7. Ultimately, it appears that the C392X mutation does not contribute to RTH by impeding nuclear export via interaction with XPO5 or XPO7, though the altered interactions of C392X TRα1 with XPO5 may in some way contribute to disease.

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Abstract 1753

Organic Extracts of Lichen *Parmelia vagans* and *Parmelia sulcata* Promote Apoptosis in Human Cancer Cells

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Meltem Guruz

Introduction: Approximately one quarter of all prescription drugs contain active ingredients of plant origins. Lichens have been historically used to treat a multitude of ailments, ranging from headaches during the Middle Ages to dressing wounds before colonial times and much of the potency of lichen relies on the secondary metabolites they produce. The purpose of this project is to analyze the antiproliferative properties of the secondary metabolites of lichens *Parmelia vagans* (Pv) and *Parmelia sulcata* (Ps).

Methods: Acetone extracts of lichens Pv and Ps were prepared using a Soxhlet extractor. The extracts were dried on a rotary evaporator, dissolved in DMSO, aliquoted, and stored at room temperature, protected from light. The antiproliferative properties of the prepared extracts were assessed using HeLa cells (Human cervical cancer) and PC-3 cells (Human prostate cancer). To examine the effect of the extracts on cell division and survival, we used a resazurin cell viability assay. Briefly, the cells were plated onto a 96-well plate and incubated overnight to let them adhere. The next day, different dilutions of the extract were added to the cells in duplicates and placed into a CO2 incubator for 24 hours. The growth media containing extract was replaced with a fresh media containing resazurin (an indicator of cell viability) and cells were incubated for another 24 hours. The changes in fluorescence were measured every 4 hours using a plate reader. The treated cells were also replated to determine the percent of live cells. Next, to understand the mechanism of cell death caused by lichen extracts, we used FITC Annexin V Apoptosis Detection Kit. HeLa and PC-3 cells were plated onto a 12-well plate and, after adherence, Pv and Ps extracts were added to the cells and placed into a CO2 incubator for 24 hours. The treated cells were then split into four aliquots and plated onto a 96-well plate. The split cells were either kept unstained, stained with FITC Annexin V, or FITC and 7-Amino-Actinomycin (7-AAD). Early apoptotic cells display phospholipid phosphatidylserine on the outer leaflet, which Annexin V binds to. Cells in late apoptotic stages will have an affinity for both 7-AAD (which binds to DNA) and Annexin V. The samples were then analyzed using flow cytometry to detect apoptotic cells.

Results: The analysis of the lichen-treated human cancer cells showed changes in cell morphology of treated cells and 60–70% inhibition of cell growth in comparison to untreated samples in cell viability assay. The apoptosis assay demonstrated positive staining of FITC Annexin V and 7-AAD in treated samples compared to untreated samples. Analysis of data from two experiments showed that 60–90% of extract-treated cells die through an apoptotic mechanism. Assessment of the antiproliferative activity of lichen extracts revealed that both extracts significantly suppress (30% - 80%) the growth of cancer cells. Moreover, the treated cell showed morphological changes that resemble an activation of apoptosis.

Discussion: Our data from the cell viability and apoptosis assays demonstrate that organic extracts of *Parmelia vagans* and *Parmelia sulcata* induce apoptosis in human cancer cells. Future experiments will be focused on the isolation and identification of the compound(s) from lichen extracts that are responsible for inducing apoptosis of human cancer cells. In summary, the lichen's secondary metabolites may hold vast medicinal potential and could be a viable source of a novel anticancer drugs.

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ASBMB

Abstract 1777

A differential role of IRSp53 isoform-S and -M in regulating cell proliferation in colorectal cancer

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IRSp53 gene encoded and translated at least four isoforms (ie. IRSp53-S, IRSp58-M, IRSp53-T, and IRSp53-L), which share the same N-terminal 511 amino-acid sequence but have a distinct 9-to-41 carboxyl-terminal amino acid sequence in each isoform. The functional role of IRSp53 in cancer cells has been implicated in the enhancement of cell motility/invasion and cell proliferation. Whether different isoform play a different role in cancer is not known. In this study, we compared the impact of IRSp53-S and IRSp58-M isoforms in cell proliferation, motility and invasion as well as integrin signaling in colorectal cancer cells. With the generation of tetracycline-off inducible IRSp53 isoform expressing SW480 cell lines, our results indicated that overexpression of IRSp58-M, but not IRSp53-S facilitates cell growth in cultured dish and soft agar as well as tumor growth in SCID mice albeit both isoforms promote cell motility in a trans-well study or wound healing assay. Online databases suggest that the level of IRSp53-S is positively correlated with better overall survivor, whereas IRSp58-M is inversely correlated with disease free survival. To confirm the positive prognostic role of IRSp53-S in colorectal cancer (CRC), immunohistochemical staining of IRSp53-S does reveal higher expression of IRSp53-S is positively correlated with better disease-free survival in cancer patients. Mechanistic study indicates that in the absence of fetal bovine serum, overexpression of IRSp58-M, but not IRSp53-S, enhances Src activity (i.e. Src Pi-Y416) and facilitates fibronectin-elicited integrin activation. Furthermore, overexpression of IRSp53-S suppresses Eps8-mediated activation of FAK, AKT and ERK in fibronectin-stimulated SW480 cells as well as cell proliferation in soft agar. Our results highlight the differential role of IRSp53 isoforms in colon cancer cells and indicate that IRSp53-S could be a prognostic factor for colorectal cancer.

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Abstract 1779

Regulation of cytokine processing by inflammatory caspases

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The mammalian innate immune system uses germline-encoded cytosolic pattern-recognition receptors (PRRs) to detect intracellular danger signals. These PRRs form multiprotein complexes called inflammasomes that activate cysteine proteases known as caspases. Canonical inflammasomes activate caspase-1, which in turn cleaves and activates inflammatory cytokines such as IL-1b and IL-18, as well as the pore-forming protein knowns as gasdermin D (GSDMD), which inducing pyroptotic cell death. Non-canonical inflammasome activation is characterized by direct detection of intracellular LPS by caspases-4/5 in human cells, which leads to their activation and cleavage of GSDMD to induce pyroptosis. Because the non-canonical pathway activates the canonical pathway downstream, it remains unclear if caspases-4/5 can directly process cytokines. Here, we used biochemistry and chemical biology to demonstrate that caspases-4/5 directly cleave IL-18. Furthermore, we show that the sequence identity of the residues adjacent to the cleavage site in IL-1b critically regulate its processing by inflammatory caspases. Our findings suggest that inflammatory caspases may have evolved to differentially regulate inflammation.

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Abstract 1789

NAK associated protein 1/ NAP1 is required for mitosis and cytokinesis through TBK1 activation

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Successful cell division is dependent on precise and timely transitions between different cell cycle phases, which is regulated by dynamic changes in protein phosphorylation. Thus, kinases play an essential role to orchestrate almost every step of cell division. Impaired or aberrant kinase activity often leads to errors in cell cycle, which consequently become the underlying cause for developmental defects or abnormal cell proliferation leading to cancer. Tank Binding Kinase 1 (TBK1) is one such kinase, which is overexpressed in certain types of cancer and regulates proper progression through mitosis. Our lab and others identified that TBK1 is activated on the centrosomes during mitosis, and its loss impairs cell division resulting in growth defects and the accumulation of multi-nucleated cells. Therefore, both proper activation and sub-cellular localization of TBK1 are essential for mitosis. Yet, the upstream regulation of TBK1 during mitosis is unknown, and we do not completely understand the function of activated TBK1 on the centrosomes. Activation of TBK1 depends on its binding to an adaptor protein which induces a conformational change and dimerization leading to trans-autophoshorylation of serine 172 on the kinase domain of TBK1. Our study objective was to identify the unknown upstream adaptor(s) and downstream substrates of TBK1 during mitosis. Using a combination of shRNA mediated knockdown cell lines, gene editing of several TBK1 associated adaptor proteins via CRISPR, and degradation tag system cell line generation, we identified the adaptor protein required for TBK1 activation during mitosis is NAK Associated Protein 1 (NAP1/AZI2). By characterizing cell division in either cancerous or near diploid cell lines, our data suggests that loss of either NAP1 or TBK1 results in the accumulation of binucleated and multinucleated cells due to several mitotic and cytokinetic defects phenocopying each other. TBK1 activation is reliant on NAP1 binding by co-immunoprecipitation experiments. NAP1 colocalizes with activated TBK1 on the centrosomes and NAP1 localization to centrosomes is abolished in binding deficient mutants. We also establish NAP1 as a cell cycle regulated protein tightly regulated by TBK1, where activated TBK1 phosphorylates NAP1 on serine 318 flagging it for ubiquitin proteasomal degradation (UPS). Further, by performing an unbiased quantitative phospho-proteomic analysis during mitosis, the substrates discovered reveal that TBK1 also regulates other known cell cycle regulating kinases such as Aurora A and Aurora B. In conclusion, our work has uncovered a novel function for the NAP1-TBK1 complex during mitosis, which is distinctive from its previously known function in innate immune signaling.

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ASBMB

Abstract 1791

The Lasting Effects of Stress Granule Dynamics: Assembly and Disassembly Upon Gαq Activation

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In the Gαq/phospholipase Cβ1 (PLCβ1) signaling pathway, activation of Gαq by neurotransmitters activates PLCβ1 which in turn raises the level of intracellular calcium. PLCβ1 is known to have a membrane-bound population involved in calcium signaling and an atypical cytosolic population that binds to stress granule (SG) proteins such as argonaute 2 (Ago2) and G3BP SG assembly factor 1 (G3BP1), among others. Upon activation, Gαq promotes the movement of cytosolic PLCβ1 to the membrane, releasing bound SG proteins leaving them free to aggregate and form stress granules. We have recently found that in PC12 cells, activation of Gαq promotes the formation of stress granules which selectively sequester two mRNAs; one that codes for a protein involved in exocrine function (Chga/b) and a subunit of ATP synthase (ATP5f1b). Here, we examine stress granule formation following stress granule proteins with single and repeated Gαq stimulation in *C. elegan* models. In *C. elegans*, a G3BP marker protein was used to analyze SG formation in neurons across different aged worms. We find that in young adult worms (Day 1), SGs completely disassemble with repeated Gαq activation. In contrast, Day 4 worms accumulate SGs and increase their speed and locomotion. In Day 8 worms, the formation of stress granules is more profound, while recovery is adversely affected. We propose that these data reflect age-related changes in SG disassembly. Taken together, our studies show a link between Gαq signaling, stress granule formation and age.

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Abstract 1804

Pseudomonas aeruginosa lipopolysaccharide presented in synthetic lipoprotein nanodiscs stimulates a robust immune response with reduced cytotoxicity

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Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen known to cause severe lung infection in immunocompromised hosts and a vaccine has yet to be developed. Gram-negative lipopolysaccharide (LPS) is highly immunogenic and a strong candidate for a vaccine antigen, however, it frequently induces adverse toxic effects in patients due to exposure to the highly toxic lipid A portion of the molecule. In a first step towards developing a robust *P. aeruginosa* vaccine, several molecular structures were created to (1) present LPS to the immune system in a physiological way, and (2) obscure the lipid A region of the molecule to reduce cytotoxicity. The first structure presented LPS in a synthetic DMPC-1D1 lipoprotein nanodisc (ND), and the second structure presented LPS bound to LPS-binding protein (LBP) either alone or tethered to a ND to avoid any inadvertent signaling caused by LBP. Immune responses were measured in human lung epithelial cells following exposure to LPS alone, LPS-containing NDs, LPS-bound LBP, or LPS-bound LBP tethered to NDs. Gene expression of 84 cytokines and chemokines were measured after 24 hours of exposure. LPS alone stimulated a significant upregulation of eight pro-inflammatory cytokines: CCL2, CCL20, CCL24, CXCL1, CXCL2, CXCL5, CXCL8, and IL-6 ($P < 0.05$). Overall, the immune responses to each of the four LPS-containing conditions were similar, with all eight genes being upregulated in each condition. The DMPC-1D1 NDs without LPS did not stimulate the same pro-inflammatory cytokine and chemokine expression, indicating that the immune responses observed were caused by LPS and not the synthetic lipoprotein itself. The LPS-bound LBP tethered to NDs displayed a slight down regulation of IL5 (1.9-fold, $P < 0.05$) and slight up regulation of CCL24 (1.7-fold, $P < 0.05$) and CNTF (1.6-fold, $P < 0.05$) but overall, the immune responses were consistent with LPS alone while observing reduced cytotoxicity. Together, these studies show that altering the biophysical presentation of LPS could provide a strategy to develop a safe *P. aeruginosa* vaccine antigen that stimulates a robust immune response and decreased cytotoxicity.

This work was funded by the Laboratory Directed Research and Development program of Los Alamos National Laboratory. This work was performed at the Los Alamos National Laboratory, which is operated by Triad National Security, LLC, for the National Nuclear Security Administration of the U.S. Department of Energy.

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Abstract 1805

Investigating the role of reduced folate carrier (RFC-1) in methotrexate resistance in model cellular systems

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Methotrexate (MTX), a common chemotherapeutic, can reduce bone mineral density in children as it negatively affects osteoblasts. Interestingly, previous work by Beane et al. in 2014 and 2016 has shown that adipose-derived stem cells (ASCs) are resistant to MTX. Thus, the mechanism of ASC resistance is of interest, and the cells may be helpful in regenerative medicine approaches to combat chemotherapeutic side effects on the skeletal system. Initially, we investigated the role of dihydrofolate reductase (DHFR) as a key player in ASC resistance. In Beane et al. 2016, we showed that DHFR knockdown in ASCs did not impact proliferation or differentiation when exposed to various MTX concentrations. Now we are exploring other candidate proteins as key players in the mechanism of ASC resistance. Preliminary searches using NLM's Gene Expression Omnibus (GEO) repository revealed that mesenchymal stem cells have significantly reduced expression of the transmembrane protein Reduced Folate Carrier 1 (RFC-1, SLC19A1 gene) compared to non-stem cell types. RFC-1 is responsible for the transport of folate into the cell and is the route in which MTX, a competitive inhibitor of folate, enters cells. Therefore, our study aims to investigate the role of RFC-1 in MTX resistance. We are using immunoblotting to semi-quantitatively assess the expression of RFC-1 in a non-stem cell model, human embryonic kidney cells (HEK293), and ASCs. Our next steps are to determine whether over-expression of RFC-1 induces ASC susceptibility to MTX, suggesting that protein plays a key role in the resistance mechanism.

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Abstract 1819

Evaluation of Imidacloprid Exposure on Cell Viability and Growth of Human Cell Lines

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This project examines if the commonly used pesticide, Imidacloprid, alters the viability and growth of human cells. Imidacloprid is a systemic pesticide and designed to enter the plant and move into growing tissues in order to prevent insect herbivory. Imidacloprid is a neonicotinoid and kills insect pests by mimicking the chemical nicotine and disrupting the function of the nervous system eventually leading to the insect's paralysis and eventually death. Imidacloprid has been detected in aquatic environments at levels above which the United States Environmental Protection Agency considers safe for aquatic life. Particularly concerning to human health, imidacloprid has also been detected in conventional fruit, vegetables, and honey intended for human consumption. In cell culture assays with a human intestinal cell line, imidacloprid was shown to be strongly absorbed. This indicates humans may be exposed through our diet and the compound absorbed into our body. Studies have indicated that this pesticide results in increased oxidative stress and increase heat shock protein levels in both invertebrate and vertebrate model systems. However, it has not been determined if exposure to varying levels of the compound results in altered cell viability and growth. Human kidney, liver, neuron and breast cancer cells will be treated with 10 uM–10 mM Imidacloprid and examined for changes in cell growth and viability using an MTS assay.

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ASBMB

Abstract 1824

TRAPPC6AΔ is a common initiator of neurodegeneration

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When WWOX is downregulated in the middle age, aggregation of a protein cascade, including TRAPPC6AΔ (TPC6AΔ), TIAF1, and SH3GLB2, starts to occur in the brain, and the event lasts more than 30 years which results in amyloid precursor protein (APP) degradation, amyloid beta (Aβ) generation, and neurodegeneration, as shown in Alzheimer's disease (AD). However, when WWOX protein is deficient in the knockout mouse, protein aggregation occurs in less than 3 weeks in the brain. Here, by treating neuroblastoma SK-N-SH cells with neurotoxin MPP+, upregulation and aggregation of TPC6AΔ, along with aggregation of TIAF1, SH3GLB2, Aβ, and tau, occurred. MPP+ is an inducer of Parkinson's disease (PD), thus suggesting that TPC6AΔ is a common initiator for AD and PD pathogenesis. Zfra, a 31-amino-acid zinc finger-like WWOX-binding protein, is known to restore memory deficits in 9-month-old triple-transgenic (3xTg) mice by blocking the aggregation of TPC6AΔ, SH3GLB2, tau, and amyloid β, as well as inflammatory NF-κB activation. The Zfra4-10 peptide exerted a strong potency in preventing memory loss during the aging of 3-month-old 3xTg mice up to 9 months, as determined by a novel object recognition task (ORT) and Morris water maize analysis. Compared to age-matched wild type mice, 11-month-old Wwox heterozygous mice exhibited memory loss, and this correlates with pT12-WWOX aggregation in the cortex. Together, aggregation of pT12-WWOX may link to TPC6AΔ aggregation which is needed for AD and PD progression.

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Abstract 1830

Seeing the Light: Inflammasome Activation in Retinal Pigment Epithelial Cells Coincides with Upregulation and Export of the Small Heat Shock Protein, Hsp27

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Age-related macular degeneration (AMD) is a neurodegenerative disease that affects the macula, the posterior portion of the retina that processes central vision. In early AMD, Drusen (yellow deposits of lipids and proteins) accumulates under the retina compromising the function of the retinal pigment epithelium (RPE). Inflammation plays a critical role in the pathogenesis of AMD, which relies upon the molecular assembly of the inflammasome complex. This complex can be mediated through different stimuli including chemical agents, crystals, and Amyloid-β (Aβ) fibrils. Our work to date has shown that the small heat shock protein, αB-Crystallin, a molecular component of Drusen, is cleaved and exported in exosomes during inflammasome activation mediated through lysosomal destabilization. However, no studies to date have investigated the role of the closely related small heat shock protein, Hsp27 following inflammasome activation. RPE cells were primed with IL-1α and the inflammasome was activated through use of the lysosomal destabilizing agent, Leucine-Leucine-OMethylesther after which exosomes were isolated from the supernatant, and total cellular protein was extracted. My work demonstrates that Hsp27 is upregulated in this model of AMD and is exported from RPE cells in exosomes as analyzed by immunoblot analysis. Concurrently, Gasdermin, a protein that promotes the release of proinflammatory cytokines, is cleaved after inflammasome activation. Alternatively, immunocytochemical analysis was performed to identify the localization of Hsp27 along with lysosomal staining. Results show abundant lysosomal aggregation following inflammasome activation and that Hsp27 does not localize to lysosomes; rather, Hsp27 expression appears to inversely correlate with lysosomal aggregation underscoring that it may promote cell survival in this model. Simultaneously, studies are underway to characterize how Amyloid-β stimulation of RPE cells promotes the expression of the NLRP3 inflammasome components and affects the trafficking of small Hsp family members, αB-crystallin and Hsp27. Recent studies have shown that apoE, the primary lipoprotein expressed in the brain and the retina, has been detected in Drusen and is involved in the upregulation of AMD markers. Therefore, an additional goal of this study is to investigate the expression of apoE following activation of the inflammasome induced by Amyloid-β. Since few therapeutic interventions exist for AMD, understanding of the molecular mechanisms in RPE cells following inflammasome activation by Amyloid-β or other stressors may provide insight to promote retinal cell viability and limit vision loss.

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Abstract 1837

The C1A Domain of PKC Confers Sensitivity to Down-Regulation

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The down-regulation of protein kinase C (PKC) enzymes is a critical degradation process to prevent aberrant PKC signaling and maintain cellular homeostasis. Although prolonged activation by phorbol esters is known to trigger PKC down-regulation in cells, the molecular mechanisms of PKC down-regulation are poorly understood. Here we take advantage of disease-associated, down-regulation-resistant mutations in protein kinase C gamma (PKCγ) to gain insight into the mechanism of PKC down-regulation. Specifically, we address why germ-line mutations in PKCγ that cause spinocerebellar ataxia type 14 (SCA14) evade quality control down-regulation despite having impaired autoinhibition. Generally, aberrant PKC with impaired autoinhibition is dephosphorylated, ubiquitinated, and degraded by the proteasome. Curiously, the majority of the approximately 50 SCA14-associated mutations cluster in the diacylglycerol-binding C1 domains (C1A and C1B) or at interfaces of the C1 domains with the kinase domain, suggesting that intact C1 domains are required for PKC down-regulation. Examination of PKC ubiquitination following phorbol ester treatment of COS7 cells overexpressing PKCγ wild-type or three SCA14-associated mutations (ΔF48 in the C1A domain, D115Y in the C1B domain, and F463L in the C-tail) revealed that whereas phorbol esters resulted in an increase in the ubiquitination of wild-type PKCγ and the D115Y and F463L mutants, the ΔF48 mutant was resistant to phorbol ester-induced ubiquitination. To test whether the C1A domain contains key ubiquitination sites, we mutated each of three potential ubiquitination sites in the C1A domain (K37R, K44R, and K61R) and assessed phorbol ester-induced ubiquitination. Whereas mutation of individual Lys did not significantly reduce ubiquitination, mutation of all three Lys residues abolished ubiquitination. These results reveal that the C1A domain is necessary for phorbol ester-induced ubiquitination, with Lys residues in the domain participating in ubiquitination mechanisms.

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ASBMB

Abstract 1846

To See or Not to See: Assessing the Implications of the P23H Rhodopsin Mutation on Retinitis Pigmentosa

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Retinitis pigmentosa (RP), caused by mutations of the protein rhodopsin, is a genetic disease of the retina in which primary rod death leads to secondary cone death. Absorption of light by rhodopsin activates the phototransduction process in which GDP replaces GTP on the G-protein. This exchange initiates the signaling pathway in the membrane of the rod photoreceptor cell that blocks Na+ and Ca2+ ions from entering the cell and results in hyperpolarization of the membrane potential. Through this mechanism, rhodopsin allows rods to pick up peripheral vision and dim light. With each rod containing hundreds of millions of rhodopsin molecules, mutations of rhodopsin inhibit these types of vision. Over 150 mutations of rhodopsin cause the gradual rod degeneration characterized by RP, but the most prominent mutation is P23H. In this mutation, histidine replaces proline in the 23rd amino acid position of rhodopsin, causing the misfolding of rhodopsin. This renders rods unable to signal light absorption, causing an initial loss of night vision followed by a secondary loss of cone structure that can result in complete blindness. Although there are currently no long-term solutions to retinitis pigmentosa, NAC Attack (a placebo-controlled clinical trial using N-acetylcysteine to slow RP), gene therapy, retinal transplantation, and other alternative methods offer potential new solutions to slow rod degeneration and prevent subsequent cone degeneration.

Tim Herman, PhD Founding Partner & Chief Science Officer, 3d molecular designs.

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Abstract 1868

Overview of protein WDR5 and its function in expounding upon anticancer therapies by delving into its structure, conservation, functions, and biological linkages

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"Moonlighting" is a phenomenon in which a protein described in one context is discovered to play functions in other, sometimes quite distinct, cellular processes. Appreciation of moonlighting has aided our growing comprehension of the complexity of eukaryotic life. It is not unexpected that proteins resist straightforward labeling, but the methods by which this happens and the consequences it has are often fascinating and deep. One such protein is WDR5, a highly conserved protein that is necessary for the appropriate control of critical cellular functions. First identified through its role in epigenetic regulation, WDR5 has been shown to participate in multiple processes, from control of protein synthesis to managing the integrity of cell division. Here, we discuss the spectrum of functions that WDR5 is able to manage in light of exceptional conservation. We discuss how WDR5 may be inhibited by small molecules due to its structure, conservation, and biological linkages, as well as how its functions may affect how these inhibitors are used as anti-cancer treatments. We will then detail the existing molecular understandings of the protein and highlight drug discovery initiatives related to WDR5 that may serve as the foundation for new anti-cancer treatments. Finally, we will use the 3-dimensional chemical viewing program JMOL and 3D printing to represent the overall structure, conformational changes, and proposed interactions of WDR5.

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Abstract 1871

The roles and potential targetability of SPOP in cancer

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Cancer is a disease hallmarked by uncontrolled cell growth. Though extensively researched, much about it remains elusive due to the complexity of its genetic, epigenetic, or signaling deregulations. In recent years, Speckle Type Poxvirus and Zinc finger (POZ) Protein (SPOP) has been identified as a cancer related protein with a promising potential for treatment. SPOP is an E3 ubiquitin ligase substrate binding subunit belonging to the Cullin-RING family of E3 ubiquitin ligases. It is in the largest subclass of E3 ligases and acts as the substrate adaptor to join with Cullin 3, creating a complex that attaches to then ubiquitinates substrates containing particular motifs. This leads to the substrate's degradation. Because of its crucial role in the proteasome pathway and its overall abundance throughout the body, when SPOP is mutated or downregulated, its effects are highly detrimental. In many types of cancer, SPOP mutations facilitate tumorigenesis. SPOP itself has three identified domains: Meprin and TRAF homology (MATH); Bric à Brac, Tramtrack and Broad complex (BTB); and BTB and C-terminal Kelch (BACK). It also has a C-terminal Nuclear Localization Sequence (NLS) at its end and a 3-box at the C-terminal of the BTB domain. Aside from one recent study, it is accepted that SPOP performs optimal ubiquitination as a homodimer, bound by the BTB domains with a Cul3 recruited to both. The MATH domains mirror each other in this formation and are better able to ubiquitinate substrates containing multiple Substrate Binding Consensus (SBC) motifs. SPOP has been most researched in regards to cancers (primarily prostate cancer), but it has also been shown to have roles in development, x-chromosome inactivation, apoptosis, DNA damage response, angiogenesis, inflammation, hemoglobin regulation, and chronic pancreatitis. SPOP mutated cancers have been shown to respond to some existing therapeutics differently, so novel treatment strategies such as targeted molecular screening have shown promise. More specialized treatments are being invented as scientists learn more about the protein and all its targets. Finally, we will use the 3-dimensional chemical viewing program JMOL and 3D printing to represent the overall structure, conformational changes, and proposed interactions of SPOP.

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Abstract 1879

Membrane Targeting of Constitutively Active Gαq Regulates Inhibition by YM-254890

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In more than 90% of uveal melanoma cases, the oncogenic driver is an activating glutamine to leucine or proline mutation at residue 209 of the α subunit of G proteins Gq or G11 (Gαq/11QL or Gαq/11QP). Up to 50% of patients experience untreatable metastases. YM-254890 is a promising inhibitor of constitutively active (CA) Gαq/11, though its mechanism of action is not well understood. Evidence supports that although Gαq/11 has 2 sites of palmitoylation that allow for association with the plasma membrane (PM), these proteins are still found in subcellular locations. We have shown that more strongly targeting GαqQL to membranes by adding an N-terminal myristoylation site renders it insensitive to YM. To further understand how PM-restricted GαqQL loses sensitivity to YM inhibition of signaling, we have generated additional membrane targeting mutants of GαqQL. Although palmitoylation at cysteines 9 and 10 of GαqQL is essential for signaling, preventing palmitoylation by mutating both cysteines 9 and 10 to serines in the context of myristoylated GαqQL does not reduce signaling, as assayed by TEAD- and SRE-dependent luciferase reporter assays in HEK293 q/11 knockout cells. Moreover, signaling by this myristoylated, palmitoylation-deficient GαqQL mutant remains insensitive to YM, indicating that introduction of a single site for myristoylation, in the absence of any palmitoylation, is sufficient to render GαqQL resistant to YM. To further define the mechanism of YM insensitivity of membrane targeting mutants of GαqQL, we have examined other CA Gα subunits, specifically Gα16 (3 sites of palmitoylation) and Gα13 (2 sites of palmitoylation), that have been mutated to introduce a YM binding site. We have generated myristoylated mutants of these Gα subunits, and they, like myristoylated GαqQL, are resistant to inhibition by YM. These experiments highlight the requirements of membrane association for signaling by GαqQL and reveal insights into the mechanism of YM inhibition. In addition, we are testing if localization is permitting the interaction of PM-restricted GαqQL with proteins or protein complexes that help facilitate YM sensitivity or resistance with the goal of further understanding Gαq cellular regulation.

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ASBMB

Abstract 1886

Human Cardiac Progenitor Cells Require ALK1 for Paracrine Signaling in Angiogenesis

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Myocardial infarction (MI) is the number one cause of cardiovascular disease mortality and is characterized by a decrease of blood flow to the heart, pathological remodeling, and irreversible cellular necrosis. Recent evidence has highlighted the protective role of transplanted cardiac progenitor cells (CPCs) in the regulation of cardiac repair, with an emerging role of CPC paracrine response and secreted proteins in this process. However, the molecular mechanisms for CPC paracrine effects on cardiac tissue function are poorly understood. Our lab has recently isolated a new class of CPCs from the human epicardium (hHiPCs). hHiPC clonal isolates are characterized by their high proliferation rate, CD90, and CD105 (Endoglin) expression. We found that Activin receptor-like kinase 1 (ALK1) is expressed in hHiPC. Using SWATH LC-MS/MS analysis of conditioned media (CM) we have found that pre-treatment of hHiPC with the ALK1 ligand, Bone morphogenic protein-9 (BMP9), increases hHiPC secretion of pro-angiogenic and BMP-regulated secreted proteins, including Sclerostin (SOST) and CD105, *in vitro*. Further, transcription of SOST and CD105 was also increased following BMP9 treatment as analyzed by RT-qPCR. To investigate this pathway's role in angiogenesis we found increased tube formation of endothelial cells and hHiPCs in the Matrigel tube formation assay in BMP9-treated CM compared to vehicle control. To further confirm the role of ALK1 in this process we used lentiviral knockdown of ALK1 in hHiPC and found significantly decreased RNA expression of CD105 and SOST following BMP9 treatment. The decrease in pro-angiogenic factors in the absence of BMP9/ALK1 signaling may suggest the potential relevance of BMP9/ALK1 signaling in cardiac progenitor cell secretome mediated repair and will be investigated using knockdown of ALK1 of transplanted hHiPC following *in vivo* MI in the future.

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Abstract 1904

Large-scale Functional Characterization of the STIM1 Calcium Binding cEF-hand Using Novel Genetic Engineering Tools

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Humans have an estimated genetic mutation rate of about 10−8 per nucleotide per generation. For an average individual, there are 40−80 de novo mutations in the genome with 1−2 of those mutations in protein coding regions. With a world population of almost 8 billion, there are thousands of possible de novo protein variants present. Most of this variation is uncharacterized, and there is an urgent need to understand the impact of these variants in human physiology and disease. Traditional methods of characterization such as transient transfection or lentiviral transduction of transgenes in mammalian cells are low-throughput, making the study of large numbers of variants particularly laborious. We have instead leveraged a multiplex high-throughput method called 'Deep Mutational Scanning' wherein hundreds to thousands of protein variants can be characterized at once. By engineering specialized 'landing pad' cells that enable a Bxb1 serine DNA recombinase to insert and stably express promoterless plasmids encoding a transgene of interest within the cell genome, we can create strict genotype-phenotype links enabling multiplex genetic assays in mammalian cells. We applied this method to study STIM1, an endoplasmic reticulum (ER) transmembrane protein critical in amplification of intracellular calcium signaling through store operated calcium entry (SOCE). Calcium signaling regulates a wide range of biological processes, including gene transcription, muscle contraction and cell death. Surface receptor activation triggers depletion of calcium in the ER which is sensed by the 36-amino acid canonical EF-hand (cEF-hand) of STIM1, initiating SOCE. A handful of known missense mutations in the STIM1 cEF-hand can cause Stormorken Syndrome from high calcium influx, or immunodeficiency from low calcium influx. However, there are many mutations within the cEF-hand observed in human exomes that are uncharacterized. To conduct a deep mutational scan of the STIM1 cEF-hand, we created and expressed a site-saturation mutagenesis library of 720 STIM1 cEF-hand variants into HEK293T cells containing the landing pad system. This pooled library of cells was assayed by a multiplex survival assay developed based on calcium-induced apoptosis, where increased calcium influx results in death of the cell. We observed variants that caused increased calcium entry deplete from the population whereas variants that caused decreased calcium entry survived. By sequencing the pooled variant library at various time points and assessing the enrichment or depletion of each variant over time, we ascribed every variant a functional score reflective of its ability to induce SOCE. This resulted in negative enrichment scores for known high calcium influx variants and positive enrichment scores for low calcium influx variants, demonstrating the accuracy of our

characterization. These functional scores can be used to interpret the impacts of uncharacterized variants in protein function, cellular dysfunction, and disease in the population. We can also use this information to learn how amino acid sequence constraints dictate how this domain exerts its calcium-sensing function.

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Abstract 1919

PTPRF negatively regulates EGFR signaling to inhibit cell migration in colon cancer

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The spaciotemporal control of cell signaling requires a balancing act of protein kinases and phosphatases. Hyperactivation of signaling downstream of receptor tyrosine kinases (RTKs) is one of the most common mechanisms leading to oncogenic transformation in numerous cancer types. Although the activation process of RTKs has been extensively studied, the inactivation mechanisms mediated by tyrosine phosphatase are less understood. Previously, we have determined the molecular mechanisms by which protein tyrosine phosphatase receptor type F (PTPRF) regulates the Wnt pathway. In this study, we investigated the functional importance of PTPRF in controlling EGFR signaling in colon cancer. Deletion of PTPRF using CRISPR/cas9 in 293T cells led to increased phosphorylation of EGFR and downstream AKT and ERK signaling upon EGF treatment. Similarly, knockdown of PTPRF resulted in an increase in EGFR activation in colon cancer cells. In addition, re-expression of WT, but not phosphatase deficient, PTPRF rescued the phenotype suggesting a phosphatase activity-dependent regulation. Co-Immunoprecipitation experiments indicated that PTPRF interacts with EGFR via its extracellular domain. However, PTPRF-mediated regulation of EGFR phosphorylation had no effect on EGF-induced receptor internalization. Functionally, knockdown of PTPRF promoted cell migration in colon cancer cells. The effect of PTPRF on controlling the specificity of signaling scaffolds downstream of EGFR is being further investigated. Taken together, our study identified PTPRF as an important regulator of EGFR signaling in colon cancer.

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Abstract 1932

AKAP Integration of Cardiac Phosphorylation and Ubiquitin Signaling

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Cardiac contraction requires the coordination of local cAMP and Ca2+ signaling events that drive the recurrent redistribution of calcium in cardiomyocytes. Excitation-contraction (EC) coupling, the process by which electrochemical stimuli are converted to contractile force within the heart, is a multistep process that includes reuptake of Ca2+ through the sarcoplasmic/endoplasmic reticulum Ca2+ ATPase (SERCA2). This critical step promotes cardiomyocyte relaxation and primes cells for the next round of contraction. Loss of functional SERCA2 protein from the sarcoplasmic reticulum in cardiomyocytes has dire consequences for Ca2+ homeostasis and is associated with cardiac hypocontractility and systolic heart failure. This work seeks to define how AKAP-directed signaling islands affect this cardiac function. We have discovered that a macromolecular complex containing enzymes of the protein phosphorylation and protein ubiquitination machinery may function synergistically to maintain SERCA2 at the sarcoplasmic reticulum in cardiomyocytes. Our molecular, cellular, and *ex vivo* calcium imaging analyses of cardiomyocytes show that the A-Kinase Anchoring protein AKAP18 sequesters the cAMP-dependent protein kinase (PKA) and the ubiquitin-specific proteinase USP4 with SERCA2. Anchored PKA phosphorylates USP4 to stimulate its deubiquitinase activity. We postulate that PKA and USP4, when associated with AKAP18, act cooperatively to protect against ubiquitin-mediated removal of SERCA2 from the sarcoplasmic reticulum.

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Abstract 1938

Surveying Cancer Cell Type Susceptibility to Ferroptotic Cell Death Via GPR68 Inhibition

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Introduction: GPR68, a G-protein coupled receptor and proton sensor, is overactivated in the acidic tumor microenvironment, promoting tumor progression. Ogremorphin (OGM), a small molecule inhibitor of GPR68, induces cell death via ferroptosis (characterized by ferrous iron accumulation). The purpose of this study was to investigate if GPR68 overexpression serves as a mechanism by which malignant cells thrive in a proton-rich tumor milieu and resist ferroptosis-mediated cell death. It was hypothesized that GPR68 expression levels would correlate with OGM sensitivity.

Methods: The investigation proceeded on three fronts: (1) OGM sensitivity was assessed using the CellTiter-Glo viability assay; cell death levels were quantitated in response to varied OGM doses. (2) GPR68 expression levels were analyzed with real-time quantitative reverse transcription PCR (qRT-PCR). (3) Lipid reactive oxygen species (L-ROS) was measured as a key indicator of ferroptosis using liperfluo staining and flow-cytometric analysis. Assays were performed across 50 different cell lines, of which 45 were immortalized cancer cell lines, and 5 were primary fibroblasts. T-tests were conducted to evaluate the significance of cell viability reductions and differences in GPR68 expression. Histograms were generated with flow cytometry data of L-ROS levels, and peak shifts between different treatment conditions were analyzed.

Results: Of the 45 immortalized cell lines, amongst those with the highest reduction in cell viability in the presence of low-dose OGM (2 μM) were the 5 brain cancer cell lines tested (Daoy, U87, U138, KR158, and GL261D) and the two lung cancer cell lines tested (A549 and H460). All 5 had statistically significant reductions in cell viability (p-value <0.001) with reductions greater than 60% relative to the control. GPR68 expression was determined by qRT-PCR in 3 cell lines, of which C2C12 was found to have the highest expression level (nearly 7 times greater than both other cell lines) and the highest OGM sensitivity (99% reduction with low-dose OGM). The remaining 2 cell lines had low GPR68 expression levels, and either no reduction (NIH3T3) or modest reduction in cell viability (C3H10T1/2) in the presence of low-dose OGM. In most cell lines, increases in L-ROS levels were associated with reductions in cell viability in response to OGM.

Conclusions: In these data, overexpression of GPR68 was associated with OGM sensitivity: 3 of the brain cancer cell lines with high OGM sensitivity are known to have high GPR68 expression, and amongst the qRT-PCR-analyzed cell lines, OGM sensitivity was highest when GPR68 expression was highest. Collectively, these data suggest that OGM-induced cell death is greatest in the setting of high GPR68 expression. Furthermore, the paralleled increase in L-ROS levels with

increased cell death levels serves as evidence that OGM induces ferroptosis-mediated cell death. This study also highlighted that brain and lung cancers, specifically glioblastoma, cerebellar medulloblastoma, and lung carcinoma, are potential therapeutic targets for OGM. Quantification of GPR68 levels by qRT-PCR in the remaining cell lines is planned. Future avenues of study include the identification of additional factors in OGM sensitivity, such as the downstream targets of GPR68, mutations that may confer OGM resistance, and the effect of intracellular acidity. Such studies will enable more precise predictions of cancers with potential for therapeutic benefit with OGM.

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Abstract 1943

The Critical Role of the Membrane Protein Ras in Cell Proliferation

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Located on the inner side of the plasma membrane, Ras proteins are molecular switches that play a critical role in signal transduction, regulating cell growth, proliferation, and differentiation (Hancock, 2003; Santos & Nebreda, 1989). Due to their crucial functions in pathways that govern the cell cycle, Ras proteins are linked to cancer. Specifically, the K-Ras isoform is one of the most frequently mutated oncogenes in human cancers, especially in lung and pancreatic cancers (Smith et al., 2001). As a small GTPase, K-Ras is activated by interaction with guanine nucleotide exchange factors (GEF) that allow GTP binding by catalyzing the release of GDP from the protein. Son-of-Sevenless (SOS) is the type of GEF most commonly associated with K-Ras. (Ehrhardt et al., 2002). Once activated, K-Ras triggers downstream proteins (Raf or PI3K) and signaling pathways that promote cell proliferation and survival (Simanshu et al., 2017). When determining the role of K-Ras in cancer, we specifically focused on the G12 mutation, a cancer progenitor in both cancer types (Hobbs et al., 2016). K-Ras mutations can induce the protein and thus the downstream pathway to be constitutively active, leading to uncontrolled cell division. Medications such as Sotorasib and Agradasib target K-Ras G12 mutations, holding the protein in its inactive, GDP-bound state and interrupting cancer progression (Skoulidis et al., 2021). The Governor's Academy Center for BioMolecular Modeling SMART team used 3D modeling technology to understand K-Ras activation, interactions with other proteins, and effector activation, as well as inhibition of mutant K-Ras by treatments. Our model highlights features including: the Switch I and Switch II regions that undergo structural changes as the protein interacts with the GDP and GTP; T35 and G60, which form hydrogen bonds with γ-phosphate of GTP and thus hold the switch regions in an active state (Simanshu et al., 2017); Y32 and Y40, residues that interact with SOS (Hall et al., 2001); and G12, the most commonly mutated residue in K-Ras-induced lung and pancreatic cancer (Santos & Nebreda, 1989).

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Abstract 1948

Phospholipase Cβ1 Induces Differentiation of Neuronal Cell Lines

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The phospholipase Cβ/Gαq signaling system plays a key but unknown role in neurological diseases. Here, we show that PLCβ1 regulates cell differentiation by three key findings. First, our previous studies and ones presented here show that differentiation of PC12, SK-N-SH, NT2 cells by various agents such as nerve growth factor, retinoic acid or AraC is accompanied by a large increase PLCβ1. Down-regulating PLCβ1 in undifferentiated cells prevents differentiation, whereas down-regulating Gαq has no effect. Second, down-regulating PLCβ1 in differentiated PC12, SKNSH or NT2 cells returns cells to an undifferentiated. Third, transfection of PLCβ1 in undifferentiated PC12 or NT2 cells allows differentiation without the need of external agents. In trying to delineate a mechanism, found noted that the level of cytosolic PLCβ1 regulates the localization of the transcription factor, Early Growth Response-1 (EGR-1), whose activity directs differentiation. Our studies support a model in which PLCβ1 regulates the state of cell differentiation through EGR-1, which may be relevant to diseases associated with PLCβ1 loss.

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Abstract 1959

A phosphorylation mimic at the Gα12 N-terminus inhibits palmitoylation and signaling to serum response factor

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The G12/13 subfamily of heterotrimeric guanine nucleotide binding proteins (G proteins) is composed of Gα12 and Gα13, which play important intracellular signaling roles including activation of serum response factor (SRF). SRF binds the serum response element (SRE) of the c-fos promoter and activates genes involved in cell growth and tumorigenic events. The G12/13 N-terminal regions have not been characterized structurally; however, previous reports indicate phosphorylation of a serine in this region of Gα12. Replacing serine-9 of a GTPase-deficient Gα12 with acidic phosphorylation mimics abolished its signaling to SRF as measured by firefly luciferase assays for SRE-mediated transcription, whereas replacement with positive charge (arginine) enhanced this response. These substitutions also inhibited SRF activation by a chimera composed of the Gα12 N-terminus attached to GTPase-deficient Gα13. In addition, we introduced negatively-charged residues adjacent to the S-palmitoylation site of Gα12 (cysteine-11) required for SRF activation; these substitutions disrupted this response. These results led us to hypothesize that mutations introducing negative charge were inhibiting palmitoylation of Gα12 at cysteine-11. To test this, we engineered N-terminal myristoylation or C-terminal isoprenylation sites in the Gα12 phosphorylation mimic mutants; both of these motifs fully rescued SRF signaling. Contrary to Gα12, signaling by Gα13 was unaffected by negatively-charged amino acids engineered adjacent to its two N-terminal sites of palmitoylation, suggesting these α subunits in the same G protein subfamily undergo distinct mechanisms of palmitate attachment. We also examined the subcellular localization of the Gα12 and Gα13 phosphorylation mimic constructs. Fractionation of cells into soluble and membranous components showed a different distribution of a Gα12/Gα13 chimera in comparison to Gα13, although both proteins exhibited robust SRF signaling. The difference in location was unaffected by phosphorylation mimics at serine-9 of the chimera. Currently, we are investigating whether engineered negative charge at the N-terminus affects Gα12 interaction with specific target proteins, and also testing kinases for the ability to phosphorylate Gα12.

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Abstract 1966

Enzyme-catalyzed proximity proteomics reveals new mechanisms of pathogenic kinase signaling in fibrolamellar carcinoma

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Fibrolamellar carcinoma (FLC) is a rare adolescent liver cancer that is not responsive to standard chemotherapy and has no FDA-approved treatment. It originates from a somatic lesion on chromosome 19 that generates a de novo fusion gene product consisting of the chaperone-binding domain of heat shock protein 40 (Hsp40/DNAJB1) linked to a catalytic subunit of PKA (PKAc) with a 14 residue N-terminal deletion. This putative oncogenic fusion enzyme is called DNAJ-PKAc. The molecular mechanism of DNAJ-PKAc action in FLC tumors is poorly understood. This work investigates pathogenic signaling mechanisms arising from the presence of the DNAJ-PKAc fusion. We have applied proximity proteomics/phosphoproteomics to identify molecular components that interact with this aberrant fusion kinase. Analysis of the mass spectrometry data revealed that DNAJ-PKAc is mislocalized from A-kinase anchoring protein (AKAP) signaling islands. This result was corroborated by live-cell photoactivation microscopy in AML12 hepatocytes, which showed wide-spread diffusion of DNAJ-PKAc away from AKAPs. Further inspection of aberrant associations of the fusion protein identified the co-chaperone BAG2, a regulator of Hsp70-mediated protein refolding and inhibitor of CHIP-mediated ubiquitination. Immunoprecipitation experiments in AML12 hepatocytes and FLC patient tissue showed co-precipitation of BAG2 with DNAJ-PKAc but not wild-type PKAc. Use of a kinase-dead mutant (K72H) demonstrated that this interaction is independent of fusion kinase activity. Furthermore, reconstitution of FLC complexes using the SpyCatcher/SpyTag heterodimerization system mimicked these findings. Future experiments will test the functional impact of BAG2 in the context of FLC pathogenesis, and whether coupling DNAJ to PKAc boosts the anti-proliferative effects of chaperone and PKAc inhibitor drug combinations. Finally, analysis of a recent proximity phosphoproteomics screen in a cellular model of FLC suggests translational regulation as a new potential tumorigenic mechanism. In sum, this study demonstrates several potential mechanisms through which DNAJ-PKAc may cause FLC. The ultimate goal of this work is to determine the molecular drivers of FLC and develop novel therapeutic opportunities for this intractable liver cancer.

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Abstract 1981

Elucidating 4R cembranoid's anti-tumorigentic mechanism in non-small cell lung carcinoma

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Non- small cell lung carcinomas (NSCLC) account for 84% of all lung cancer diagnoses, with an average 5-year survival rate of 26% due to less sensitivity to traditional treatments such as radiation and chemotherapy. Chemotherapies such as cisplatin and carboplatin are platinum-based and cause adverse effects on non-cancerous healthy cells. Therefore, the need for non-toxic treatments is crucial for a better prognosis in patients. In our search for these anticancer agents, our group tested and demonstrated that (1S,2E,4R,6R,-7E,11E)-2,7,11- cembratriene-4,6-diol (4R), a cembranoid extracted from Nicotiana tabacum, is non-toxic and has anti-proliferative and anti-tumorigenic activity in NSCLC, while not affecting non-cancerous cells. Our studies aim to elucidate if 4R causes cell death in NSCLC via autophagy or apoptosis, thus describing 4R's anti-tumorigenic mechanism. 4R has been shown to negatively modulate the pro-survival and pro-proliferation alpha 7 nicotinic acetylcholine receptor (α7nAChR). To confirm 4R's negative modulation of nicotinic receptors, we evaluated the LYNX-1 expression (an α7nAChR negative modulator) in LLC and A549 cells after treatment with 4R. Results demonstrated that after 4R treatment, LYNX-1 expression significantly increases compared to its vehicle (*$P < 0.0383$ and *$P < 0.0349$, respectively). Based on our data, we hypothesize that 4R, a negative modulator of nicotinic signaling, will inhibit molecular cascades involved in survival and proliferation, inducing cell death. To test this hypothesis, we compared the levels of the α7nAChR in NSCLC cell lines to primary normal lung cells (MRC5) by Western blot (WB). Results showed significant overexpression of the α7nAChR in A549 and Lewis Lung Carcinoma (LLC) lung cancer cell lines when compared to MRC5 ($P < 0.0001$). To test if 4R reduces NSCLC viability by autophagy, we evaluated the protein expression of LC3B, a microtubule-associated protein that mediates autophagy. No significant difference in LC3B expression was observed in LLC and A549 cells treated with 4R compared to their vehicle. We also studied by WB Akt expression and activation, an essential protein kinase for cell survival downstream of the α7nAChR, in both LLC and MRC-5 cells. Results demonstrated that 4R-treated cells significantly decreased phospho-S473 and total Akt (**$P < 0.0078$ and **$P < 0.0061$, respectively) while not affecting MRC-5 cells. Furthermore, we studied the pro-apoptotic protein BAD and observed a significant decrease in phospho-S112 when treated with 4R (*$P < 0.0453$) without affecting MRC-5 cells. In summary, our results suggest that in NSCLC, 4R induces apoptosis rather than autophagy by inhibiting α7nAChR signaling through the upregulation of LYNX-1. This modulation decreases the activation of pro-

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survival pathways and promotes the activation of pro-apoptotic proteins such as BAD. Further evaluation of apoptotic signaling and caspase activity in LLC and A549 cells will help us confirm our hypothesis. Thus, we propose that 4R could serve as a novel, non-toxic, adjuvant treatment against NSCLC.

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Abstract 2012

Mediator kinase inhibition suppresses chronic inflammation in Down syndrome

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Down syndrome (trisomy 21) is a genetic disorder caused by the presence of an extra copy of chromosome 21. One of the hallmarks of this disorder is immune dysregulation; individuals with trisomy 21 are disproportionately affected by chronic inflammation and autoimmunity, resulting in high rates of diseases like type 1 diabetes and Celiac disease. These co-morbidities largely stem from the presence of four interferon (IFN) receptor subunits on chromosome 21. Chromosomal triplication causes overexpression of these subunits, resulting in hyperactive IFN signaling. Notably, partial knockout of IFN receptors increases viability of trisomy cells, suggesting that treatments that target the IFN response may be useful in reducing immune dysregulation and improving the prognosis of Down syndrome individuals. Involved in the regulation of the IFN response are Mediator kinases CDK8 and CDK19. CDK8 and CDK19 are essential for driving transcriptional changes in response to IFNγ. Their involvement has introduced the possibility of using Mediator kinase inhibition (via selective inhibitor Cortistatin A (CA)) to dampen hyperactive IFN signaling in individuals with Down syndrome. Moreover, studying the effects of Mediator kinase inhibition in T21 and D21 cells will enable us to better understand the roles of CDK8 and CDK19 in Down syndrome under basal and IFN-induced conditions. Recent metabolomics and RNA-Seq data produced by our lab show that Mediator kinase inhibition results in significant metabolic changes that influence autophagy, mitochondrial function, nucleotide biosynthesis, and fatty acid metabolism; furthermore, numerous links to the mTOR pathway were evident, implicating CDK8 and CDK19 in these processes. To further investigate the role of Mediator kinases in these processes, we are completing follow-up experiments involving flow cytometry and Seahorse XF analysis in cells from sibling-matched D21 and T21 individuals. We have identified several pathways and cellular functions that are impacted by Mediator kinase inhibition, in agreement with our metabolomics and transcriptomics data. For example, glycolysis and mitochondrial electron transport is negatively affected by CDK8/19 inhibition and autophagy is sensitive to CDK8/19 kinase function. Interestingly, T21-specific effects (vs. D21) are

observed, suggesting genotype-specific regulation by CDK8 and/or CDK19. Collectively, these experiments illustrate how Mediator kinases affect physiological processes under basal and IFN-stimulated conditions. They also implicate Mediator kinases as "druggable" targets to mitigate chronic inflammation and other co-morbidities associated with Down syndrome.

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Abstract 2026

Epigenetic and transcriptional regulation of CXCR4 by circadian clock regulated miRNAs in human monocyte derived macrophages

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Monocyte trafficking is imperative in maintaining optimal functioning and tissue homeostasis wherein; the interactions between C-X-C chemokine ligands (CXCLs) and receptors (CXCRs) are a key driving force. CXCR4 and CXCR7 are implicated in several autoimmune diseases, lifestyle disorders and malignant carcinomas. Paradoxically, time-dependent migration of circulating monocytes often points towards a circadian control in chemokine expression that is pivotal in maintaining monocyte plasticity and pathogen scavenging. We have observed that CXCR4 receptor is expressed in synchrony with Brain and muscle ARNT-Like 1 (BMAL1) and Circadian Locomotor Output Cycles Kaput (CLOCK). However, the clock-mediated control affecting CXCR4 expression in monocytes under circulation lacks conclusive evidence. Herein, we hypothesize a circadian control of CXCR4 in circulating monocytes by BMAL1/CLOCK heterodimerization and its subsequent binding to the enhancer box (E-box) in its promotor region. In this study, we identify a novel mechanism by which BMAL1/CLOCK and microRNAs (miRNAs) cooperatively modify CXCR4 mRNA, mediating its expression and thereby leading to a time-dependent migration of circulating monocytes. We used two different in silico approaches to identify miRNAs having (E-box) binding sites in their promotor region and targeting 3′ untranslated region (3′UTR) of CXCR4 mRNA using promotor mapping, molecular docking techniques, miRbase and CoGeMiR databases. Our study reports that both strategies were highly effective in selecting a group of 8 miRNAs (miR-139, miR-548, miR-95, miR-206, miR-185, miR-372, miR-9 and miR-302) implicated as epigenetic regulators. Using shRNA-induced BMAL1 knock-down in human monocyte derived macrophage (THP1) cells, we had recorded lowered expression of miRNAs (miR-548, miR-95, miR-185) thus providing evidence on clock mediated regulation of the same. Simultaneous lowering of CXCR4 expression provided further confirms our hypothesis on its epigenetic and transcriptional regulation in circulating monocytes. Subsequently, we also had observed a significant downregulation of genes (CXCR4-Src-Ras/Raf-ERK1/2) involved in migration and metastasis pathways. Our data on oleic acid-induced THP1 monocyte-derived macrophages (MDMs) corroborate our findings wherein; a significant upregulation of BMAL1 had led to an equivalent increase in CXCR4 expression. Taken together, perturbations in the circadian clock affects the chemokine receptor-ligand crosstalk, piloting toward an obstructive monocyte migration. Findings also highlight the importance of BMAL1-mediated targeting of CXCR4/

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CXCL12/ACKR3 axis (in autoimmune disorder), CXCR4/CXCL12 axis (in lifestyle disorder) and CXCR4/CXCR7/CXCL12 axis (in cancer metastasis).

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Abstract 2033

Fine-tuning GPCR-mediated neuromodulation by biasing signaling through different G-protein subunits

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GPCRs mediate neuromodulation through activation of heterotrimeric G-proteins (Gαβγ). Classical models depict that G-protein activation leads to a one-to-one formation of Gα-GTP and Gβγ species. Each of these species propagates signaling by independently acting on effectors, but the mechanisms by which response fidelity is ensured by coordinating Gα and Gβγ responses remain unknown. Here, we reveal a paradigm of G-protein regulation whereby inhibitory GPCR responses are biased to favor Gβγ over Gα signaling in neurons. We have identified a G protein regulator that binds tightly to Gαi-GTP to preclude its association with effectors (adenylyl cyclase) and, simultaneously, with Regulator-of-G-protein-Signaling (RGS) proteins that accelerate deactivation. As a consequence, Gαi-GTP signaling is dampened whereas Gβγ signaling is enhanced. We show that this mechanism is essential to prevent imbalances of neurotransmission that underlie increased seizure susceptibility *in vivo*. Our findings reveal an additional layer of regulation within a quintessential mechanism of signal transduction that sets the tone of neurotransmission.

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Abstract 2058

A combined proteomic and genetics approach to identify *in vivo* kinesin functional dynamics

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Kinesins are a broad class of motor proteins that function primarily, but not exclusively, to carry cargoes toward the plus ends of microtubules. Various *in vitro* biochemical and *ex vivo* cell-based approaches have been utilized to probe kinesin activity and dynamics, such as autoinhibition when inactive, binding cargo via the kinesin cargo-binding domains or engagement with the microtubule substrate via the kinesin motor domain. However, few studies have addressed the functional and biochemical interplay between cargo binding and substrate engagement once each domain is engaged with a binding partner. Here, we used the model system *C. elegans* and its well-established genome and proteome to probe the *in vivo* functional dynamics of KLP-4, the worm homolog to human kinesins KIF13A and KIF13B. KLP-4 is a neuronal kinesin expressed primarily in head and nerve cord command interneurons. To identify potential interacting proteins and cargoes, we performed LC-MS/MS shotgun proteomics from mixed-stage worms containing an endogenous CRISPR tagged 3x-FLAG version of KLP-4. This approach identified 331 novel proteins, 32.6% of which are involved in protein production, suggesting a role of KLP-4 in local protein synthesis in neurons. Included, but not unexpected our proteomics approach were 16.9% cytoskeletal related including both alpha and beta-tubulin. We therefore complimented our proteomics approach with modulating the stability of microtubule substrates in *C. elegans*. Using an overactive version of KLP-4, we have identified a specific genetic interaction between klp-4 and the microtubule acetyltransferase atat-2. When combined, these animals display behavior hallmarks of precocious synapse formation in command interneurons consistent with the early onset of synapse development. The results from our combined approach suggest a functional relationship between microtubule stability and the role of KLP-4 in neuronal development and maintenance.

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Abstract 2064

Modulation of TAL1 Gene Expression in T-ALL JURKAT Cells Using Synthetic Zinc Finger DNA-Binding Proteins

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive blood cancer that makes up to 15% of ALL cases in children and up to 25% of ALL cases in adults. A subset of T-ALL, about 5%, is characterized by high level expression of the transcription factor TAL1. TAL1 is involved in the specification of myeloid cells and positively regulates the differentiation of red blood cells. Aberrantly high-level expression of TAL1 in T-ALL is mediated by a mutation of a cis-regulatory DNA element that creates one or multiple binding sites for the transcription factor Myb. The presence of the Myb binding sites creates a super-enhancer (SE) that drives high-level expression of TAL1. We generated a zinc finger (ZF) DNA-binding domain targeting the Myb binding site in JURKAT cells, a T-ALL cell line harboring the Tal1 associated mutation. The 8 ZF protein targets a 24 bp sequence overlapping the Myb binding site. *In vitro* studies demonstrated that the ZF protein interacts with the Myb DNA-binding site with high affinity. Delivery of the ZF protein to JURKAT cells either via virus mediated delivery of a 8ZF-Myb expressing gene or via direct protein transduction reduced expression of Tal1 and cell proliferation of JURKAT cells. We generated additional 8 ZF-Myb expression constructs in which Myb is fused to a peptide facilitating delivery or to a repression domain (KRAB) expected to confer long-term silencing of the Tal1 gene.

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Abstract 2077

The Epidermal Growth Factor Receptor variant III (EGFRvIII) induces the assembly of a unique intracellular compartment with mixed early endosomal and endoplasmic reticular nature

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The Epidermal Growth Factor (EGF) Receptor (EGFR) variant III (vIII) is expressed in many cancers such as glioblastomas (GB) (40% cases), head and neck squamous cell carcinoma (30%), prostate cancer (20%) and breast cancer (20%). It is found only in tumors and not normal tissue making it an excellent candidate for targeted therapy. This variant of EGFR has an in-frame deletion in the extra-cellular domain which creates a junction between amino acids 5 and 274 and an introduction of a novel glycine residue at the junction. This deletion of 267 amino acids affects the ligand (EGF) binding domain. EGFRvIII is considered to be constitutively active; however, the molecular basis for such behavior are not fully understood. Here, we investigated the characteristics of EGFRvIII trafficking and signaling. Our work showed that cells transfected with EGFRvIII triggered the assembly of a unique and previously unnoticed large, intracellular compartment which colocalize (nearly 100%) with EEA1 (early endosome marker) and PDIA3 (ER marker). This indicated an ER and endosomal mixed nature of the intra-cellular compartment. In addition, we showed that cells expressing EGFRvIII displayed weak binding to tetra-methyl-rhodamine labeled EGF. To test the role of ligand binding in compartment assembly, cells expressing EGFRvIII were treated with growth factors. We showed that these compartments disassembled when serum/EGF-starved and reassembled when stimulated with growth factors as a function of dosage and time as early as 4–8 hours of stimulation. These intracellular compartments resembled the effects of constitutively active Rab5 Q79L mutant. Thus, to further investigate, we co-transfected Rab5 WT or Rab5 S34N (dominant negative) along with EGFRvIII. Our results showed that Rab5 WT colocalized with EGFRvIII-containing structures while Rab5 S34N interferes with their assembly indicating the role of endosomal fusion in compartment formation. We speculate that these intra-cellular compartments displaying early endosomal nature could be sites for endosomal signaling. Additionally, endocytosis deficient kinase dead mutant of EGFRvIII (EGFRvIII-K721M) expresses no intracellular compartments suggesting the importance of internalization of EGFRvIII to trigger assembly of the compartments. Further, to test if the formation of compartments is solely a characteristic of EGFR activation, we transfected cells with a constitutively active kinase mutant of EGFR (EGFR-L858R) but did not observe the assembly of analogous intracellular structures. This suggests that EGFRvIII has unique properties that lead to the generation of an abnormal membranous structure. Since EGFRvIII can heterodimerize with EGFRWT, it will be crucial to understand the role of co-expressing EGFRWT and characterize compartment assembly and downstream signaling events. This study will help decipher the molecular mechanism behind EGFRvIII subverting intracellular physiology and laying the foundation for future novel cancer therapies.

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Abstract 2092

Dissecting the molecular basis for the modulation of neurotransmitter GPCR signaling by a Gαi-binding protein

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It is well-established that activation of heterotrimeric G-proteins (Gαβγ) by G-protein-coupled receptors (GPCRs) stimulated by neurotransmitters is a key mechanism underlying neuromodulation. Much less is known about how G-protein regulation after receptor-mediated activation contributes to neuromodulation. We have recently identified a regulator of Gi proteins that shapes GPCR inhibitory neuromodulation and underlies neurological processes affecting pain and seizure susceptibility. The molecular basis of this mechanism remains ill-defined because the structural determinants of this regulator responsible for binding Gαi subunits and regulating G-protein signaling are not known. Here, we combined hydrogen-deuterium exchange mass-spectrometry, protein folding predictions, bioluminescence resonance energy transfer assays, and biochemical experiments to identify specific amino acids required for Gαi binding. Surprisingly, our results support a model in which this G-protein regulator undergoes a significant conformational change to accommodate Gαi binding. Using cell-based assays, we validate that the specific amino acids required for G-protein binding are also essential to regulate, differentially, Gαi-GTP and free Gβγ signaling upon neurotransmitter GPCR stimulation. In summary, these findings shed light onto the molecular basis for a post-receptor mechanism of G-protein regulation that fine-tunes inhibitory neuromodulation.

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Abstract 2110

Intersection of Trained Immunity and Metabolism in Macrophages Through SETDB2

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The mammalian immune response is mediated through two complementary systems. These include innate immunity which recognizes invading pathogens with little specificity and historically thought to have no memory component; The second is the adaptive immune response which recognizes invading pathogens with high specificity and thought to be unique because of its robust and specific memory component. However, recent studies have demonstrated innate immunity also has a memory component. Several pro-inflammatory signals such as microbial cell derived β-glucan, have been identified as initial "triggers" that train macrophages to respond to a later secondary challenge. Unlike Adaptive immunity, this memory response is mediated through epigenetic reprogramming. SETDB2 is a chromatin associated protein with high homology with H3K9 methyltransferases and negatively regulates the proinflammatory response to pathogen exposure, in fact, the proinflammatory phase is prolonged in macrophages lacking SETDB2. Objective: To evaluate whether SETDB2 plays a role in the trained immunity.

Methods: BMDMs were isolated from mouse bone marrow and trained with β-glucan for 24 hr. Following training, cells were washed and cultured for 6 days with GM-CSF, followed by secondary stimulation with LPS for 6 h. All animal procedures were approved by Institutional Animal Care and Use Committees (IACUCs) at Johns Hopkins University.

Results: We compared training effects in WT or SETDB2 deficient BMDMs, where the secondary response in the SETDB2-KO cells was dramatically reduced. RNA-sequencing analysis showed ~2200 differentially regulated genes between WT vs. SETDB2-KO BMDMs. Further k-means clustering, revealed a dynamically changing profile of clustered transcripts. Cluster 1 contains 475 protein coding genes that exhibited a positive correlation with the presence of SETDB2 while 857 genes in cluster 2 correlated negatively with SETDB2 in response to β-glucan training. These differential genes were selectively associated with several metabolic and inflammatory pathways including: glycolysis, hypoxia, and some inflammatory pathways in cluster 1 while, cluster 2 was enriched for genes included in Interferon gamma and alpha regulated pathways. ATAC-sequencing further showed differential open and closed chromatin regions in the promoters of genes associated with glycolytic and pro-inflammatory pathway. To assess if these changes were associated with SETDB2 enzymatic function, we evaluated the expression of genes associated with cluster 1 and cluster 2 pathways in knock-in mice harboring a change in two amino acid that would eliminate binding of the methyl co-factor SAM and inhibit its enzymatic function. Interestingly, LPS and β-glucan induced glycolytic pathway genes from cluster 1 was

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blunted in the SETDB2 knockout but were induced normally in the SETDB2 knock-in BMDMs. In contrast, Interferon responsive genes from cluster 2 that were negatively regulated by β-glucan in WT BMDMs were not suppressed in BMDMs from either SETDB2-KO or knock-in mice. These results suggest that SETDB2 may regulate different immune response pathways by two different molecular mechanisms, one requiring its enzyme activity and the other that does not. Conclusion: Overall, our preliminary data suggest that SETDB2 is key regulator of trained immunity in macrophages that connects metabolic flow to the innate immune response and future work will further elucidate the mechanisms by which SETDB2 contributes to different pathways involved in the training response.

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Abstract 2112

Investigating the Interaction between MK-STYX and Vimentin

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MK-STYX [MAPK (mitogen-activated protein kinase) phosphoserine/threonine/tyrosine-binding protein) is a pseudophosphatase belonging to the MAPK phosphatase (MKP) subfamily within the protein tyrosine phosphatase (PTP) superfamily. The absence of a histidine and critical cysteine in the signature active motif render MK-STYX catalytically inactive, earning it the prefix pseudo. A mass spectrometry study revealed that MK-STYX and the cytoskeletal protein vimentin are binding partners. Vimentin is expressed in the early stage of neuronal development, contributes to organelle organization, and localizes at the perinucleus, mitochondria, and golgi. It has been reported that the downregulation of vimentin decreases neuritogenesis. Previous research revealed that MK-STYX increases neurite outgrowth in rat pheochromocytoma PC-12 cells and rat hippocampal primary neurons. This interaction of MK-STYX and vimentin and the role of MK-STYX in neurite formation point towards a possible relationship between the two proteins that may contribute to neuritogenesis. Our goal is to further confirm and characterize the interaction between MK-STYX and vimentin. HEK-293 cells were cotransfected with the following mammalian expression vectors: GFP and mCherry, GFP-MK-STYX and mCherry, GFP-MK-STYXactive and mCherry, mCherry-vimentin and GFP, GFP-MK-STYX and mCherry-vimentin, or GFP-MK-STYXactive and mCherry-vimentin followed by western blot analysis. Vimentin expression was increased in the presence of MK-STYX. To further characterize the dynamics between MK-STYX and vimentin, we performed colocalization experiments and used an fluorescence microscopy to analyze them. The conditions were GFP, mCherry, GFP and mCherry, GFP-MK-STYX, mCherry-vimentin, GFP and mCherry-vimentin, GFP-MK-STYXactive and mCherry-vimentin, or GFP-MK-STYX and mCherry-vimentin. Higher rates of partial colocalization were observed in GFP-MK-STYX and mCherry-vimentin conditions (≥75%) than in the mCherry-vimentin and GFP condition, confirming the pattern observed in our pilot colocalization experiments. All instances of colocalization were observed in the cytoplasm and future studies will investigate vimentin localization at the perinucleus, mitochondria, or golgi in the presence of MK-STYX and MK-STYX (active mutant), which has phosphatase activity. Taken together the colocalization pattern of MK-STYX and vimentin and the increase of vimentin in the presence of MK-STYX support the hypothesis that MK-STYX and vimentin are interacting and together may serve as important players in neuritogenesis.

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Abstract 2113

TBLR1, a canonical RARa cofactor, mediates retinoic acid resistance in acute myeloid leukemia by preventing RARa recruitment

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Most acute myeloid leukemias (AMLs) are unresponsive to retinoic acid (RA)-induced myeloid differentiation, a process controlled by the RA-inducible transcription factor RARa. In the canonical RA signaling pathway, TBLR1 (also known as TBL1XR1) is recruited by DNA-bound RARa. This recruitment represses the ligand-independent transcriptional activity of RARa and contributes to the optimal RA response. Unexpectedly, here we uncovered a novel function of TBLR1 in inhibiting the RA response in AML. Mechanistically, we show that in RA-resistant AML cells, TBLR1 is recruited to a RARa-independent repression complex and plays a role in preventing RARa recruitment, thereby precluding the regulatory ability of RA and leading to RA resistance. In RA-sensitive AML cells, RARa has bound to DNA, and TBLR1 acts as the canonical RARa corepressor compatible with the RA response. We also validated the RA response inhibition function of TBLR1 in primary AML. In summary, our results reveal a novel function of TBLR1 required for RA resistance in AML. We show that this function is active in primary AML and targets the recruitment of RARa, a rate-limiting step in RA responsiveness in AML. RA differentiation therapies may thus benefit from inhibiting this function to enable the recruitment of RARa. To our knowledge, this is also the first study showing that a canonical nuclear receptor (NR) corepressor can not only prevent ligand-independent transcription but also prevent ligand-dependent NR-mediated transcription through the formation of distinct NR-dependent and NR-independent repression complexes.

This work was supported by the Start-up Fund from the Department of Pharmacology and Physiology Saint Louis University School of Medicine, the President's Research Fund of Saint Louis University, and the Siteman Investment Program (SIP) Award from the Siteman Cancer Center at Barnes-Jewish and Washington University School of Medicine.

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Abstract 2119

In vitro characterization of novel mechanisms involved in BRAF activation and regulation

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Rapidly Accelerated Fibrosarcoma (ARAF, BRAF, CRAF) Kinase is central to the MAPK Signaling Cascade (RAS-RAF-MEK-ERK). According to the accepted model, inactive RAF kinase is monomeric, autoinhibited, and cytosolic while activated RAF is membrane recruited via RAS-GTP. During activation, RAF is dimerized and phosphorylated. Phosphorylation plays a crucial role in regulating BRAF, however, key details are still missing. Using a chemical biology approach to target this complex biological system, we seek to exert spatial control using the genetically encodable Chemically Induced Dimerization (CID)-BRAF; CID-BRAF exploits the FRB-rapamycin-FKBP system to selectively recruit BRAF to the plasma membrane. Under serum starvation conditions, membrane recruitment by CID leads to activation of BRAF. This suggests that mechanisms other than RAS binding can also relieve the autoinhibitory interactions exerted by intramolecular interactions. It is well documented that active and inactive BRAF have differential (activating and inactivating) phosphorylation sites. To this end, we report the characterization of a novel phosphorylation site, identified using the CID-BRAF system. This novel phosphorylation site is in proximity to a 14-3-3 binding motif. Mutational analysis suggests the novel site inversely affect the binding of 14-3-3, therefore indirectly regulate the enzyme activity and drug sensitivity of BRAF. Further characterization of this novel site will provide additional mechanistic insight to the regulation of BRAF and facilitate the development of next generation inhibitors of dysregulated BRAF.

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Abstract 2125

PKN1 is a novel therapeutic target to prevent graft-vs-host disease after allogeneic hematopoietic cell transplantation

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The study objective is to identify protein kinase N1 (PKN1) as a novel therapeutic target for optimal control of graft-versus-host disease (GvHD) in mice and humans. Our group was the first to demonstrate that targeting JAK1/2 using small molecule inhibitors, baricitinib (BARI) and ruxolitinib (RUX), prevents and treats GVHD while enhancing multi-lineage hematopoietic reconstitution and graft-vs-leukemia (GvL) effect in mouse models of allogeneic hematopoietic cell transplantation (allo-HCT). However, unlike BARI, RUX fails to completely prevent GvHD even though it does significantly reduce clinical GvHD in our mouse models of allo-HCT. Furthermore, we reported that BARI reverses established GvHD not only by modulating immune cells to prevent further tissue damage to GvHD target organs but also by directly promoting GvHD-damaged tissue repair via EGFR signaling. To elucidate the mechanism underlying the superiority of BARI over RUX, we performed both mouse and human kinome analyses. We found that BARI, but not RUX, is a potent inhibitor of both murine and human PKN1, suggesting that the superiority of BARI to RUX in preventing and treating GvHD may also result from the off-target effects of BARI on PKN1 in addition to its known inhibitory effects on JAK1/2. While the function of PKN1 in GvHD remains unexplored, PKN1 has been suggested as a protein kinase in signaling pathways involving S1PR1 and LFA-1 (αLβ2 integrins) that are critical for immune cell adhesion/trafficking and the balance of regulatory T cells (Tregs) and effector T cells (Teffs). The candidates include the S1PR1/PPP1R14A, LFA-1/STK4, and LFA-1/RPH3A pathways. To narrow down the candidates for more focused investigation, we examined if OTS167, a PKN1 inhibitor, alters the downstream signaling of these candidate pathways. We found that OTS167 significantly reduces the phosphorylation of PPP1R14A and STK4 in murine T cells activated by anti-CD3/CD28 antibody-coated beads in a dose-dependent manner. Of note, RPH3A or pRPH3A is not detectable in T cells, which is consistent with Expression Atlas (www.ebi.ac.uk/gxa/home). In addition, we found that *in vivo* administration of OTS167 reduces GvHD in a mouse model of allo-HCT ($p < 0.05$, a pool of 2 independent experiments). Furthermore, since PPP1R14A is downstream of S1PR1, we examined if S1PR1-deficient T cells cause less GvHD. We found that allo-HCT recipient mice transplanted with S1PR1 KO T cells demonstrate significantly improved overall survival ($p < 0.05$, $n = 7$ or 8 per group). These data suggest that PKN1 and S1PR1/PPP1R14A (and also LFA-1/STK4 which is currently under investigation) are critical components in inducing GvHD. PKN1 has also been known to mediate the TLR2-NF-kB signaling pathway initiated by cardiolipin (CL) to upregulate the expression of MHC II and costimulatory molecules on antigen presenting cells (APCs). Activated PKN1 by cardiolipin-TLR2 induces the nuclear localization of NF-kB. We previously reported that BARI significantly reduced the level of MHC II and CD80/CD86 on recipient APCs after allo-HCT compared to RUX. Thus, we hypothesized that BARI-induced blockade of PKN1 would reduce the nuclear localization of NK-kB in APCs. Indeed, we found that the nuclear localization of NF-kB in CL-activated APCs was significantly reduced by BARI compared to the control groups (vehicle and RUX; $p < 0.001$). All of these data support our hypothesis that PKN1 is a novel therapeutic target molecule along with previously known JAK1/2.

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Abstract 2128

High Molecular Weight Hyaluronan Accumulates Chronically after Myocardial Infarction

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Background: The extracellular matrix (ECM) is a complex network of various macromolecules. It provides structural and functional support to the heart. Following a myocardial infarction (MI), there is a rapid turnover of matrix proteins in the cardiac ECM. Some components accumulate in the ECM to contribute to scar formation, while the role of other ECM constituents remains unknown. One of the most abundant components of the cardiac ECM is hyaluronan (HA), a glycosaminoglycan. Our data shows that HA accumulates after MI; however, the role that accumulation of HA plays in ventricular remodeling is unknown. In addition, the relative size of HA influences its biologic function. Hence, understanding the size of HA found in the ECM post-MI is key to understanding the role HA plays in ventricular remodeling.

Hypothesis: High molecular weight (HMW) HA accumulates in the infarct zone after MI. Methods and

Results: We subjected wild-type male and female mice to non-reperfused myocardial infarction and isolated HA from the infarct zone and remote zone; we assessed hearts at 1 week and 4 weeks post-MI ($n = 6$). Semi-quantitative assessment of HA showed an increase in HMW HA in 4-week infarct zone compared to naïve hearts ($p < 0.0001$), 1-week infarct zone ($p = 0.0046$), and 4-week remote zone ($p = 0.0007$). There was a smaller increase in the remote zones at both time points and 1-week infarct zone.

Conclusion: Our data indicate that HMW HA accumulates chronically after MI with slight increases acutely after MI.

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Abstract 2131

The higher integrin α5β1 levels in the male kidney cortex indicate a potential reason for the better urine concentration ability in men than women

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In recent years, more attention was given to understanding the sex difference in the physiology and pathology of diseases. Multiple studies have reported sex differences in renal physiology based on basic renal structure, which showed larger renal cortex volume and proximal tubules in males than that in females. Renal proximal tubules are the major mediators of solute reabsorption from the glomerular filtrate and AQP1-mediated water reabsorption. Studies also showed a higher urine concentration ability in males compared to females that correlated with AQP1 levels. Recently we demonstrated that lower integrin β1 levels inhibited urine concentration ability. Since the sex differences in integrin expression of the kidney and its relation to renal physiology are unknown, sex-specific expression of integrin subunits in the kidney cortex was investigated. Various integrin subunits were compared between male and female kidneys by immunoblotting and qRT-PCR. Higher integrin α5 and β1 protein levels were observed in the male kidneys compared to female kidneys; integrin α5 (male 1.07 ± 0.603, female 0.471 ± 0.170; $p < 0.05$) and integrin β1 (male 1.86 ± 0.852, female 0.891 ± 0.320; $p < 0.05$). There were no significant differences in integrin α1 (male 0.9112 ± 0.374, female 0.782 ± 0.082) and αV (male 1.04 ± 0.654, female 0.979 ± 0.285) protein levels between male and female kidneys. The mRNA levels for integrin β1 (male 1.02 ± 0.178, female 0.67 ± 0.188; $p < 0.05$) also showed higher in male kidneys compared to female kidneys. Experiments are progressing to determine the mRNA level of other integrin subunits in the kidney. Decreased integrin α5 and AQP1 levels in the proximal tubule epithelial cells by the deletion of integrin β1 were recorded by immunofluorescence. Further studies are required to correlate the role of specific integrin subunits and sex differences in kidney functions.

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Abstract 2139

The role of collagen in the progression and migration of thyroid cancer

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The BRAFV600E oncogenic mutation is the most common genetic alteration in thyroid cancer, particularly in papillary thyroid cancer (PTC). BRAF-driven tumors have been shown to contain a large proportion of fibroblasts and a higher population of macrophages. The recruitment of fibroblast is associated with a fibrotic and reactive tumor stroma consisting of fibrillar collagen deposits and increased expression of lysyl oxidase (Lox). Data has shown an increase COL1A1 and LOX expression correlates with mutant BRAF expression and poorer overall survival rate was observed in patients with overexpression of COL1A1 and LOX. In this project we will begin to define the role of collagen in the extracellular matrix (ECM), and how this modulates tumor cells. We believe that investigating the molecular mechanisms behind the role of COL1A1 and LOX in an *in vitro* culture model can help understand the interactions between tumor cells, collagen and stromal cells. We established murine cancer-associated fibroblast tumor cells lines, MCAFs, with knockdown of either Col1a1 or Lox. CRISPR/Cas9 was used to stably knockout Col1a1 or Lox. Knockdown of Col1a1 and Lox will be confirmed by Western Blot and real-time qPCR. We will determine the role of Col1a1 and Lox in fibroblast growth and proliferation through cell proliferation and migration assays. This project establishing stable cell lines and clones of MCAFs with Col1a1 and Lox knock-down will be foundational for future studies to investigate how the ECM and stromal affect cellular transformation, metastasis and the behavior of tumor cells.

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Abstract 2152

Synthesis of Fluro-estrone analogs targeting Hepatocellular Carcinoma

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Hepatocellular Carcinoma (HCC) is the third leading cause of deaths worldwide. It is associated with chronic liver diseases such as chronic hepatitis, and chronic alcohol consumption. Epidermal growth factor receptor (EGFR) tyrosine kinase (TK) has been identified as a molecular target for designing potential drug candidates. EGFR TK is a validated drug target for the treatment of multiple cancers including HCC. Recent development in our group revealed novel estrone analogs possessing C-17 side chain have shown potent antiproliferative activity against HCC and HCC resistant to Erlotinib. A pervious report from the Halaweish lab depicts the impact of cucurbitacin inspired estrone analogs (CIEA) against a hepatocellular carcinoma cell line. They found two of these planned analogs, MMA102 and MMA132 (Enantiomers at carbon 19, Figure 2A), act as dual inhibitors against phosphorylating pathways in the Epidermal Growth Factor Receptor (EGFR) and MAPK pathways. Moreover, it has been shown that these two analogs likewise have phosporylating pathways [OTRSS1] against a safe HCC cell line to erlotinib, the standard anticancer treatment (an EGFR inhibitor) for HCC. Investigation of the construction of MMA102 and MMA132 demonstrates a potential imperfection. An α,β-unsaturated ketone exists in the side chain of the side chain. This species is very helpless to digestion by Michael addition, prompting a 1,4 addition product. We accept the planar idea of the ketone is to some extent liable for natural movement of the compound because of direction of the hydroxyl gathering, and loss of this bond could prompt diminished action, especially in the safe cell line. The double bond in any design could bring about Michael addition, and subsequently planarity is possibly hard to keep. To battle this, we propose functionalizing the double bond. Primer information has been directed involving OpenEye® Molecular Modeling in which 110 estrone analogs with either - CN or -F usefulness at the β position to the ketone (Figure 1A) were docked inside various proteins in the EGFR cascade. Results demonstrate that specifically those analogs with cyanide moieties are even more frequently to improve binding in numerous proteins. Analog, NA85 (Figure 1B), shows high binding scores to Several EGFR proteins. In store for this task, we wish to proceed with these computations by running molecular dynamic simulations. We likewise plan to blend NA85 and other high scoring compounds, as well as test them *in-vitro* on both sensitive and resistant HCC cell lines.

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Abstract 2167

Dual T Cell Receptor Fusion Constructs (TRuCs) for the Immunotherapy of Pediatric Solid Tumors

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Introduction Chimeric antigen receptor (CAR) T cell therapy is a clinically approved and effective treatment for heme malignancies but has limited success against solid tumors. The lack of response is a result of insufficient target antigens and physical and immunosuppressive barriers. We propose that T cells that simultaneously target both an antigen on the tumor surface and in the tumor microenvironment (TME) will overcome these limitations. B7-H3 is the selected antigen target on the tumor surface as it is uniformly expressed on tumor cells. Fibronectin extra domain B (EDB) is the TME target and a splice variant of fibronectin. Additionally, we wanted to test if a TRuC that harnesses the autoregulatory and signaling power of the T cell receptor (TCR) would function like CARs. Material and Methods Generation of plasmids PCR amplification expanded a linearized viral vector and inserts encoding for EDB and B7-H3 single-chain variable fragments (scFvs). DNA was stained, isolated with gel electrophoresis, exercised, and dissolved. In-fusion cloning generated the B7-H3 and EDB retrovirus vectors. Stellar cells were mixed with the infusion reaction, incubated, and colonies were selected the following day. DNA was PCR amplified and analyzed via gel electrophoresis with primers specific to the inserts. Successful colonies for each construct were expanded. Transfection 293T cells were transfected with the expanded DNA and incubated. After 2 days, the cell supernatant was harvested, filtered, and snap frozen. Generation of TRuCs Human peripheral blood mononuclear cells (PBMCs) were stimulated and fed. On day 2, the T cells were transduced with retroviral supernatant. Dual TRuCs were produced by pooling the two monospecific supernatants. Transduction was checked via flow cytometry 5 days after transduction. Coculture Non-transduced (NT) T cells and TRuCs were cultured on their own or with A549, A549FN1 −/−, or A549B7H3−/− tumor cells. After 2 days, the supernatant was collected and interferon gamma (IFNy) was measured. MTS TRuCs, NT T cells, and A549, A549FN1−/−, A549B7H3−/− tumor cells were plated at 4:1, 2:1, 1:1, and 1:2 effector to target (E:T) ratios. After 5 days, the media and effector T cells were removed. Remaining cells were treated with MTS reagent and incubated for 2 hrs. Living cells were measured at an absorbance of 492 nm. Results Expression The B7-H3 TRuC had 80–90% transduction efficiency while the EDB TRuC had 55–90% efficiency. The dual TRuC successfully expressed both B7-H3 and EDB scFvs with a transduction of 75–95% and 80–90% respectively. Anti-tumor activity Both dual and B7-H3 TRuCs had complete tumor depletion of the A549 wild-type (wt) cell line at a 2:1 E:T ratio. The EDB TRuC was less effective. Both B7-H3 and dual TRuCs killed all A549FN1−/− cells. EDB TRuCs showed slight anti-tumor activity against the A549B7H3−/− cell line, while the dual TRuCs were able to completely kill the tumor cells. The same patterns of response were seen in the 4:1 E:T ratio. Activation All TRuC constructs have high IFNy production with the wt cell line. Only the EDB and dual TRuCs were activated by the A549B7H3−/− tumor cells. Similarly, the A549FN1−/− only activated the B7-H3 and dual TRuCs. Conclusion The dual transduction method resulted in dual targeting T cells with the same transduction efficiency as monospecific TRuCs. The primary successes of the dual TRuCs were complete tumor killing across all cell lines regardless of knockout status and antigen specific activation.

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Abstract 2169

The novel adrenal Cushing's syndrome mutant PKAc-W196G retains binding to type I regulatory subunits

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Mutations in the catalytic subunit of protein kinase A (PKAc) drive adrenal Cushing's syndrome, a disorder of chronically elevated stress hormone levels. Previous reports have demonstrated disrupted binding of Cushing's PKAc mutants to their regulatory subunits. Additionally, overproduction of stress hormone by two of these mutants, PKAc-L205R and PKAc-W196R, has been shown to require subcellular mislocalization of the kinase away from holoenzyme complexes. Here, we report on the discovery and functional characterization of a previously unidentified Cushing's mutant, PKAc-W196G. Patient tumor samples were genotyped and deep sequenced within exons 6–8 of PRKACA. From ten samples, we detected an individual with the prominent L205R kinase variant and two patients with a T > G missense mutation at base 589, resulting in a glycine substitution at tryptophan 196 in the protein. Expression of PKAc-W196G in adrenal cells caused elevated stress hormone production as compared to the wild-type kinase (PKAc-WT), consistent with this mutation driving the disease. Furthermore, staining of patient tissue demonstrated mislocalized PKAc signal in both L205R and W196G tumors. In contrast to PKAc-L205R, which does not discriminate between cytosolic and nuclear localization, PKAc-W196G was largely excluded from the nucleus. Immunoprecipitation and live-cell photoactivation experiments in adrenal cells demonstrated diminished binding of PKAc-W196G to type II regulatory subunits (RII), as expected. However, these experiments revealed the surprising result that PKAc-W196G retains normal interactions with the type Iα regulatory subunit (RIα), as well as the endogenous PKA inhibitor PKI. Intrinsic dynamics simulations were performed to understand how such binding selectivity is achieved by this mutant kinase. Initial results suggest that the presence of RII disallows sidechain interactions on the catalytic subunit, thereby disrupting proper coordination and decreasing contact area between the two subunits. Thus, a novel mutant kinase found in adrenal Cushing's syndrome drives the disease despite retained associations with RIα and PKI. Future studies are needed to understand how specific subpopulations of PKA contribute to adrenal function in both healthy individuals and Cushing's syndrome patients.

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Abstract 2178

Understanding TAOK1 dysfunction in neurodevelopmental disorders using human stem cell derived models

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Smita Yadav

Thousand and one amino acid kinase 1 (TAOK1) encodes a serine/threonine protein kinase that is important for normal neuronal migration and dendrite arborization. Clinical data and exome sequencing studies reveal TAOK1 as one of the genes associated with both autism and NDD. Recent findings from our lab suggest that autism-associated catalytically dead mutations in TAOK1 can aberrantly trap it at the plasma membrane causing exuberant membrane protrusions. In this study, we generated human WTC11 iPSC lines harboring a catalytically dead, NDD associated heterozygous (TAOK1S111F/+) and homozygous S111F mutation (TAOK1S111F/S111F) along with their isogenic control WTC11 iPS cell line. Dorsal forebrain neural progenitor cells (Pax6+, Nestin+) were derived for each line using two independent differentiations. The effect of S111F mutation in TAOK1 on NPC morphology, proliferation and motility was investigated using live cell imaging, Ki67-CycA staining and wound healing assay respectively. Preliminary results indicate a multi-lobed nucleus, increased proliferation and a decreased migration capability of NPCs in the homozygous TAOK1(S111F) mutant NPCs compared to the control. Future experiments include investigating the contribution of TAOK1 in early neurodevelopment using human stem cell derived 3D cortical organoids and neurons differentiated from NPCs.

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Abstract 2194

Evaluating the Resistance of Adipose-Derived Stem Cells to the Chemotherapeutic Agent Methotrexate

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Louise Darling

Methotrexate (MTX) is a common chemotherapeutic drug that prevents the cellular proliferation of cancer cells by inhibiting an essential protein for DNA synthesis, dihydrofolate reductase (DHFR). Despite being a commonly used agent, the non-selectivity of the drug can cause impairment of healthy progenitor cell differentiation and proliferation, which medically means that patients, including children being treated for acute lymphoblastic leukemia (ALL), are at a higher risk of osteoporosis (Mandel, et. al, 2004). Interestingly, our previous work showed that adult adipose-derived stem cells (ASCs) not only resist but also proliferate and retain multi-differentiation potential after being treated with MTX (Beane, et al., 2014). Our current approach utilizes fluorescence spectroscopy-based proliferation and viability assays to quantify the response of ASCs and a model non-stem cell (HEK-293s) to various clinically-relevant concentrations and time courses of MTX treatment. We expect that ASCs will proliferate and retain high viability after being with MTX at concentrations below 500 μM for 48 h. The expected threshold concentration level for HEK-293 cells is anticipated to be 2.5 μM, as other non-stem cell types show susceptibility to MTX at concentrations of 2.5 μM after 48 h of exposure (Beane et al, 2014).

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Abstract 2197

Control of Brm Expression in the Epigenetically Distinct SW13 Cell Line Subtypes

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The SW13 human adrenocortical carcinoma cell line exists in proliferative SW13- and metastatic SW13+ subtypes which interconvert by epigenetic mechanisms in an epithelial to mesenchymal transition (EMT) process. The SWI/SNF chromatin remodeling complex controls inducible gene expression and appears to play a key role in the transition between subtypes. This large 2 MDa complex has a modular structure which provides flexibility to mediate changes in gene expression during differentiation and development and is considered essential for cell survival. Two major complex variants, SWI/SNF A and B, have been defined and exact subunit composition appears to change as stem cells differentiate during development. Regardless of subunit composition, SWI/SNF complex function depends on ATPase activity and cancer therapies targeting these subunits have been developed. As profound changes in gene expression are required for the subtype transition, we hypothesize that the SWI/SNF complex composition varies between the two SW13 subtypes. Work here focuses on the expression of Brm (SMARCA2), one of the canonical SWI/SNF ATPase subunits and several accessory subunits. Data presented here provide the initial characterization of the SWI/SNF complex composition in each SW13 subtype.

Methods: Western blot analysis of nuclear extracts from both SW13 subtypes was performed to quantitate expression of subunits characteristic of SWI/SNF A and B respectively. Immunoprecipitation experiments were performed to demonstrate an association between subunits. qPCR experiments were performed to assess changes in Brm expression.

Results: Expression of subunits characteristic of SWI/SNF A and B appear to be altered in the two SW13 subtypes. Specifically, the highly proliferative SW13 subtype expresses higher levels of ARID2 suggestive of a SWI/SNF B-like complex while the subtype with greater metastatic potential expresses higher levels of DPF3 suggestive of a SWI/SNF A-like complex. Immunoprecipitation experiments reveal that core subunits physically associate in both subtypes. Secreted components from one cell type appear to control the expression of Brm which may initiate the switch between proliferative and metastatic subtypes.

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Conclusions: Our data suggest that the composition of the SWI/SNF complex may differ between SW13 subtypes. These data extend the role of the SWI/SNF complexes beyond differentiation and development to rapid phenotypic transitions seen in epigenetic remodeling of gene expression.

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Abstract 2202

CTBP1 Mutation Dysregulate Wnt Pathway and Affect Neuronal Development

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De novo mutations in the CTBP1 (C-terminal Binding Protein) gene are associated with neurodevelopmental defects leading to ataxia, hypotonia, intellectual disabilities, and tooth/enamel defects in children. Our preliminary studies comparing transcriptome data of control and mutant neurons derived from induced pluripotent cells revealed 145 genes that are differentially expressed. Of the 145 genes, there are several that interfere with the Wnt signaling pathway, an essential signal transduction pathway that regulates numerous cellular processes. Wnt plays a significant role in neuronal survival, neurogenesis, regulation of synaptic plasticity, and control of axon and dendrite development. In our previous study, we observed that the mutant neurites were thinner than the control. Our hypothesis is that the CTBP1 mutation causes dysregulation of the Wnt pathway, although it may not be fully suppressed or inhibited. Therefore, the addition of a Wnt inhibitor should affect neurite development during neuronal differentiation in the mutant compared to the control. We used XAV-939, a WNT signaling inhibitor that induces Axin2-dependent-catenin degradation. The addition of the Wnt inhibitor significantly affected neurite development in the mutant cells compared to the control. This result supports our hypothesis that Wnt pathway is dysregulated in CTBP1 mutant neurons and affects neurite growth.

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Abstract 2203

Kinetic Assay of cyclic di-GMP signaling in *Paracoccus denitrificans*

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Erik Yukl

Cyclic diguanosine monophosphate (c-di-GMP) signaling is well known for regulating diverse functions like motility, virulence, and biofilm formation in gram-negative bacteria. Intracellular levels of this important secondary messenger are controlled by the activities of diguanylate cyclases (DGC), which synthesize it thereby increasing concentrations of c-di-GMP, and phosphodiesterase (PDE) that degrade it. Increasing levels of c-di-GMP are associated with an increase in biofilm formation in most species. However, this trend appears to be reversed in *Paracoccus denitrificans* where a hyper-biofilm phenotype is observed for a mutant strain lacking both annotated DGC genes DdgcA/dgcB. Further, a heme nitric oxide/oxygen binding protein (H-NOX) gene is adjacent to the dgcA gene and a Dhnox strain is found to be biofilm deficient, suggesting that H-NOX may inhibit c-di-GMP synthesis by DgcA. H-NOX proteins have been found to regulate c-di-GMP metabolism and biofilm formation in other species in a NO-dependent manner. Presumably, NO binding to the heme cofactor alters the H-NOX conformation and its resultant interactions with downstream proteins. Intriguingly, the proximal histidine residue found in other H-NOX homologues that coordinates the heme cofactor is replaced by proline in *Paracoccus denitrificans*, leaving some question as to how this could function as an NO-responsive regulator of c-di-GMP. Here, we describe the heterologous expression of both H-NOX and DgcA. As predicted, *P. denitrificans* H-NOX purified without bound heme. We evaluate the DGC activity of DgcA in the presence and absence of H-NOX using HPLC and show that DgcA is an active DGC enzyme that is not significantly inhibited by recombinant H-NOX. Finally, ELISA was used to determine total c-di-GMP production in WT, Dhnox, and DdgcA/dgcB *P. denitrificans* strains. A rather startling finding is that c-di-GMP levels appear to increase in the DdgcA/dgcB strain, suggesting that this organism may have alternate pathways for c-di-GMP synthesis. This work is expanding our understanding the diversity of mechanisms for c-di-GMP metabolism among bacterial species and the role they play in regulating biofilm formation.

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Abstract 2216

Characterization of the cvn7 Conservon Operon in *Streptomyces coelicolor*

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Streptomyces coelicolor is a gram-positive bacterium that is commonly found in soil. This bacterium has proven its use and importance with its antibiotic production and other secondary metabolites. In addition, it has also served as an advantageous organism to study prokaryotic growth and development. Recently, *S. coelicolor* research has focused on a conserved system of thirteen operons, called the conservon. Each operon consists of a gene encoding a probable Ras-like GTPase in addition to three or four other genes. Some conservon operons within *S. coelicolor* have proven to be interesting due to the effects of mutating such genes, causing *S. coelicolor* to produce altered pigmentation patterns of antibiotics when exposed to other species of actinomycetes. Within our lab, we have found the genes within the cvn7 operon to be of particular interest, as not much literature has been published about this operon yet. We have conducted work on four of the five genes from this operon: cvnB7 (SCO6795), cvnC7 (SCO6796), cvnD7 (SCO6797), and cvnF7 (SCO6798). We obtained the transposon mutated versions of each gene within cosmid 1A2 of *Escherichia coli*. These strains of *E. coli* were later conjugated with wild-type *S. coelicolor* selecting for the apramycin resistance gene of the transposon. Apramycin-resistant colonies were picked and plated onto media containing either kanamycin or apramycin. We chose apramycin-resistant, kanamycin-sensitive candidates that indicated a double homologous recombination event had occurred resulting in the disruption of each gene of interest. We identified six candidates for SCO6795, five for SCO6796, nine for SCO6797, and five for SCO6798. These strains are being grown on plates that feature all candidates from each mutated gene, including wild type for phenotypic reference. The macroscopic colony phenotype of each mutant differs from that of the wild type. We plan to continue this project by examining the microscopic phenotype of each mutant in comparison to the wild type. We will then analyze phenotypic changes when the *S. coelicolor* mutants are grown in close proximity to other bacterial species. By studying the effects of altering the genes within the cvn7 operon, we could possibly discover how these alterations can benefit our understanding of microbial interactions, metabolic processes, and even antibiotic production.

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Abstract 2224

Multiple Sclerosis and its Inequitable Effect on Marginalized Backgrounds

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Multiple Sclerosis (MS) is a neurological disorder in which the immune system attacks the myelin sheath. This causes inflammatory lesions in the brain and spinal cord, disrupting nerve impulses and causing symptoms such as sensory and motor deficits, weakness, numbness, etc. Through literature review and conversing with multiple experts on MS, it was found that immunological and hormonal differences, and socioeconomic factors play an imperative role in the gender bias of MS, and is being studied further in the following review. Several factors distinguish the presentation of MS in women from its presentation in men. In women, MS has an earlier onset, higher prevalence (sex ratio 3:1), more frequent relapses, and more inflammatory lesions. This suggests a gender bias potentially associated with genetic or hormonal factors. MS lesions occur when T-cell lymphocytes and macrophages infiltrate damaged areas and develop scars impeding nerve cell transmission in the brain. Immune response in adult females is generally stronger and more adaptive than male immune responses, and have a higher predisposition to autoimmune and immune-mediated diseases like MS. These immunological differences and their role in forming inflammatory lesions in the brain could be a key factor in the differences in susceptibility and progression in women with MS. Studies have shown that certain sex hormones have neuroprotective effects and remyelination potential for patients with MS. Varying levels of these hormones in men and women can serve as biomarkers for the lack or abundance of certain symptoms. MBP has an unequivocal link to MS and implications of gender bias based on its interactions with immune cells is also hypothesized to be an early indicator of MS and aid in the progression of the disease. MBP is a structural protein responsible for the maintenance and re-formation of the myelin sheath. MBP has 6 isoforms in humans, 3 of which are widely researched: 17.5 kDa, 18.5 kDa, and 21.5 kDa. 18.5 kDa is the most prevalent isoform. 18.5 kDa is distinct in that it has post-translational modifications that cause charge variants, C1-C8. A study has revealed that MBP's cross-reactivity with immune cells shows potential implications of gender bias. Digital modeling was utilized to generate a 3D visual of MBP in order to better understand its pivotal role in MS. Additionally, studies find that certain systemic socioeconomic factors may not account for clinical study results from women of color. Moreover, research has shown that the factors that contribute to the appearance of MS vary between different racial backgrounds. Particularly, decreased vitamin D production in people of color has shown that its supplementation could protect individuals against MS. This demonstrates that individuals of color have predetermining factors pertaining to MS that healthcare providers should monitor. Although research on the correlation between MS and its effect on people of color is not widespread, the research that is currently present can continue to evolve, and from there, treatments along with more research for MS that consider individuals of color can eventually play an integral contributing role in the studies of MS and beyond. The results of this review have uncovered several factors correlating to gender bias, racial inequity, and proteomic factors related to MS that can aid in furthering relevant studies that account for such disparities.

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Abstract 2225

Investigating Gemcitabine Resistance Mechanisms and Novel Drug Combinations in Pancreatic Ductal Adenocarcinoma

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Christopher D'Inzeo

Despite great effort in the development of treatment strategies for pancreatic ductal adenocarcinoma (PDAC), response rates of current gold standard chemotherapies such as gemcitabine remain low. Additionally, resistance to such chemotherapies proves a rising challenge in the treatment of many cancers, including PDAC, and knowledge is lacking on the molecular mechanisms of this chemoresistance. This study aims to increase the understanding of chemoresistance mechanisms and explore novel combination therapies for chemoresistant PDAC through the development of a gemcitabine-resistant PDAC cell line. To quantify intrinsic chemoresistance, PANC 04.03 cells were grown in gemcitabine concentrations ranging from 0.35 nM to 350 uM and cell viability was assessed using the Cell Titer Glo assay. Preliminary results show low levels of cell death beginning at 350 uM gemcitabine, suggesting higher levels of intrinsic resistance than expected. Future work will first examine higher concentrations of gemcitabine to obtain GI50 concentrations, followed by treatment of cells with low gemcitabine concentrations to begin the development of acquired chemoresistance. Following the development of gemcitabine resistance, the role of guanine quadruplexes (G4s) in development of chemoresistance will be explored via visualization and quantification through immunofluorescence experiments on parental and resistant cell lines. If there is a correlation between G4s and chemoresistance development, then cells will be treated with combination therapies consisting of drugs that target G4s in combination with other well-established therapies. Taken together, this work will provide a model to study gemcitabine resistance in PDAC, increase the understanding of the molecular mechanisms of G4s in chemoresistance, and open avenues for novel treatments of chemoresistant PDAC.

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Abstract 2226

The Role of Adenosine in Brain Injury Induced Apoptosis of the American Cockroach

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The emerald jewel wasp, *Ampulex compressa*, envenomates its host, *Periplaneta americana* (American cockroach), directly at the cerebral ganglia. After envenomation, a state of hypokinesia is induced and the cockroach becomes compliant, however, the cockroach can continue to feed, groom, and stand when provoked. The venom does not show any signs of being necrotic or cytotoxic, and although the venom has a long lasting effect, the cockroach resumes normality after several days. A previously generated venom apparatus transcriptome and venom proteome outlined many signaling pathways that are possibly targeted by the venom. One of these venom components is adenosine deaminase (ADA), which may antagonize purinergic signaling in the brain. ADA has been shown to catalyze the deamination of adenosine to inosine, which may be key to understanding the mechanisms of the venom. Literature has suggested that excessive adenosine signaling post-injury can lead to cellular stress and eventual apoptosis. Adenosine is a ligand to the adenosine receptor (AdoR); in cell cultures, cells that express AdoR will undergo apoptosis when exposed to high concentrations of adenosine. Both adenosine and adenosine monophosphate (AMP) are ligands to AdoR, as well as substrates to venom ADA. The venom ADA and the cockroach brain AdoR have been cloned and expressed and it has been shown that the presence of venom ADA weakens the adenosine induced calcium signaling *in vitro*. We hypothesize that at the site of envenomation, venom ADA is neuroprotective by attenuating brain injury induced signaling.

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Abstract 2231

Investigation of Synergistic Combinations of Chemotherapy Drugs for the Treatment of Oral Cancer

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Makayla Oby

Oral cancer is one of the most common forms of cancer among developing countries but despite current efforts, the survival of oral cancer has remained constant. Current research on oral cancer is centered on clinical studies focused on single drugs or very few combinations of the most common therapeutics historically used to treat oral cancer. However, there is a major gap in the literature about combinations of drug treatments for oral cancer. The goal of this study is to explore combination treatments and assess whether any synergistic effects can be produced. To accomplish this, two oral squamous cell carcinoma (OSCC) cell lines, Cal-27 and OECM-1 from the tongue and the oral cavity, respectively, will be used to investigate the efficacy of single drugs as well as combinations of drugs from a wide array of chemotherapy agents currently used to treat oral cancer as well as other forms of cancer. Thus far, Cell Titer Glo assays have been performed in triplicate for both Cal-27 and OECM-1 to analyze their viability in the presence of four drugs used to treat other forms of cancer with different mechanisms of action: PD0, a MEK inhibitor, IBET-762, a BET inhibitor, ABT-199, a Bcl-2 inhibitor, and JQ1, a BRD4 inhibitor. GI50 values were gathered from each assay. The same procedure was followed for 4 drugs currently being used to treat oral cancer including Vincristine, a microtubule formation inhibitor, Gemcitabine, a DNA polymerase inhibitor, Hydroxyurea, a ribonucleotide reductase inhibitor, and Docetaxel, a microtubule depolymerization inhibitor. Cal-27 presented higher GI50 values than OECM-1 for IBET-762 and JQ1 at 1.52 mM and 1.64 mM, respectively. The GI50 values for OECM-1 treatment with IBET-762 and JQ1 were lower at 1.07 mM and 1.21 mM, respectively. Both cell lines were found to have similar resistance to PD0, around 1.05 mM, and ABT-199, around 1.02 mM. High GI50 values across the cell lines indicate that both Cal-27 and OECM-1 are resistant to the four drugs that were tested. When testing current oral cancer drugs, in both cell lines, Vincristine produced GI50 values around 1.25 μM, Gemcitabine produced GI50 values around 0.1 mM, and Docetaxel produced GI50 values around 0.05 μM. Hydroxyurea produced a GI50 value of 180 mM for Cal27 and the GI50 was not reached for OECM-1. Further testing at higher concentrations will be completed to determine the GI50 for Hydroxyurea in OECM-1. In the future, Cal-27 and OECM-1 will be treated with new combinations of drugs currently used to treat oral cancer and drugs not currently used for oral cancer but used to treat other forms of cancer. Cellular responses will then be assessed to determine if any combinations are synergistic. The data generated can aid researchers in understanding how different OSCC cells respond to drug combinations, which may lead to more successful clinical treatment options in the future.

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Abstract 2256

LncRNAs in regulation of inflammation and macrophage activation in human

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Long noncoding RNAs (LncRNAs) are emerging as major players in regulation of gene expression, cell signaling, and metabolism. Studies from our laboratory demonstrated that lncRNA HOTAIR, a well-known transcriptional repressor, regulates cytokine expression and glucose metabolism during inflammation in macrophage via NF-κB activation. In a recent study, to identify lncRNAs regulating inflammation signaling in human, we performed RNA-seq in lipopolysaccharide (LPS) stimulated THP1 derived macrophage (THP1-Mϕ). Based on the RNAseq analysis, along with cytokines, and many protein coding genes, we discovered a series of novel lncRNAs that highly up- or downregulated upon inflammation in macrophages, suggesting their potential roles in inflammation and macrophage activation. We termed these lncRNAs as LinfRNAs (Long-noncoding inflammation associated RNA). The structure and function of most of the hLinfRNAs (human LinfRNAs) are unknown and are being investigated in our lab. Our study demonstrates that inhibition of NF-kB suppressed the LPS-induced expression of most hLinfRNAs, suggesting potential regulation via NF-kB activation. Antisense-mediated knockdown of hLinfRNA1 (ADORA2A-AS1) suppressed the LPS-induced expression of cytokines and pro-inflammatory genes such as IL6, IL1β, and iNOS expression, suggesting critical roles of hLinfRNA1 in cytokine regulation and inflammation. Further functional characterization of selected lncRNAs, elucidating their RNA-protein interaction profiles and roles in cell signaling and metabolism are in progress. Overall, we discovered a series of novel hLinfRNAs that are potential regulators of inflammation and macrophage activation and may be linked to inflammatory and metabolic diseases.

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Abstract 2258

In Situ Targeting of Adrenal Cushing's Syndrome PKAc-L205R Disease Variant

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Maryanne Kihiu, Mitchell Omar, Kyung-Soon Lee, Katherine Forbush, John Scott

Adrenal Cushing's syndrome is an endocrine disorder where adrenal cells release an excess of the stress hormone cortisol. Primary symptoms include midsection weight gain and moon face. These are accompanied by comorbidities such as hypertension, type II diabetes, and neuropsychiatric disorders. Current treatments include surgery followed by radiotherapy and administration of steroidogenesis inhibitor drugs. Yet, this treatment regimen is available to only a subset of patients, leaving others with no curative options. Patients with active disease have severely diminished quality of life and up to four times higher mortality rate. Thus, there is need for developing other effective treatments. Here, we investigate a peptide that is based on the sequence of protein kinase A inhibitor (PKI), as a potential treatment approach for specific disease types of adrenal Cushing's syndrome. Whole exome sequencing studies of patient adrenal tumor samples revealed that adrenal Cushing's is associated with mutations in the catalytic subunit of the cAMP-responsive holoenzyme, protein kinase A (PKA). The predominant mutation is the L205R mutation which occurs in more than 45% of adrenal Cushing's cases. This mutation abrogates interaction with all known regulatory proteins of the kinase including PKI. In addition, our phospho-proteomic studies indicate that PKAcL205R may exhibit an altered substrate specificity with preference for proline-directed substrates. This is in strong contrast with PKAcWT which disfavors proline-directed substrates as a typical serine/threonine kinase. Bioinformatic and biochemical studies suggest that proline in the position following the phosphorylated serine or threonine (P + 1) may play a significant role in the interactions of PKAcL205R with its substrates. Given that regulation partners are PKA pseudo-substrates, we speculated that the substitution of PKI isoleucine in the P + 1 site, to proline, might re-establish interaction with PKAcL205R, rescuing inhibition. Studies with this modified PKI, henceforth referred to as PKIPro, show surprising results where PKIPro leads to PKAcL205R depletion in a dose-dependent manner. Drug studies with the potent proteasomal inhibitor MG132 suggest that this PKIPro-induced depletion is independent of the proteasome. Furthermore, immunofluorescence studies show that wild-type PKI shuttles PKAc out of the nucleus, but PKIPro is ineffective in PKAcL205R nuclear export. We speculate that the PKIPro-PKAL205R complex is unable to go through the conformational

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changes necessary to unmask the nucleus export signal (NES) required for nuclear export. Future studies will test this hypothesis and measure the effect of PKIPro on cortisol production.

Shurl and Kay Curci Foundation University of Washington- The Department of Pharmacology.

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Abstract 2292
Conditional knockout of E2F5 results in metastatic tumors after long latency

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The development of breast cancer can be due to altered regulation of mammary gland development. A better understanding of normal development can thus reveal potential mechanisms for how normal cells are reprogrammed to become malignant. E2Fs1-4 have demonstrated roles in normal mammary development while little is known for the repressor E2F5 due to early lethality. A combination of scRNAseq and predictive gene expression signatures strongly suggested a role for E2F5 in both mammary development and as a tumor suppressor in breast cancer. Thus, we hypothesized that a loss of E2F5 would result in altered mammary development and accelerated tumor development in other mammary tumor models. To test this hypothesis, we generated mice with a mammary epithelial specific loss of E2F5. As predicted, loss of E2F5 in the mammary epithelium resulted in a delay in ductal outgrowth with knockouts lagging being controls by 25% at 4 weeks of age. Given that the structure of the mammary gland was preserved, it was not surprising that the knockout mammary epithelium eventually completely filled the fat pad and the ductal network was indistinguishable from controls. Cycling through pregnancy, lactation and involution revealed minor delays in the apoptosis and remodeling associated with the cessation of lactation. Interestingly, when mice were examined at one year of age, the loss of E2F5 was associated with a surprising overgrowth of the mammary glands. Based on this, we revised our initial hypothesis that E2F5 would accelerate tumor formation of another model system and instead hypothesized that loss of E2F5 alone would result in tumor formation. After an extended latency, loss of E2F5 in the mammary epithelium resulted in mammary tumor formation. The resulting tumors were highly varied histologically, with patterns that included microacinar, papillary and EMT among others. The tumors were highly metastatic and we noted metastatic lesions in the lungs, liver and lymph nodes. Given the extended latency of the model system, we transplanted the E2F5 conditional knockout tumors into syngeneic recipient mice. We repeatedly observed lymphatic metastases in addition to the primary tumor. Testing for the ability to enrich this lymphatic metastasis phenotype, we repeatedly implanted lymphatic metastases into the mammary fat pad. After four rounds of transplantation this resulted in over 80% of primary tumors developing lymphatic metastasis. RNAseq and Whole Genome Sequencing has shown several interesting mechanisms. This includes elevated CyclinD1 activity with E2F5 loss, but similar to HER2/Neu tumors, the E2F5 tumors are likely dependent upon CyclinD1 since CyclinD2 and D3 are not upregulated. While these studies are in a mouse model, we predicted E2F5 activity in human breast cancer using a transcriptomic signature built in human mammary epithelial cells. This revealed that low E2F5 activity was associated with poor patient survival.

Moreover, given the lymphatic metastasis phenotype and the role of lymph node (LN) metastasis as a diagnostic in human breast cancer, we examined TCGA data with LN status. This revealed a significant association between low E2F5 activity and lymph node metastasis. Taken together, these data reveal that mammary epithelial specific loss of E2F5 alters mammary development and results in tumors that form after a nearly 2-year latency. Importantly, this a new model with lymphatic metastasis and clear parallels to human cancer.

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Abstract 2311

Shaking up Spheroids: The role of Hsp27 and aB-crystallin in anoikis inhibition during ovarian cancer spheroid formation

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Anoikis is a form of programmed cell death that cells undergo as they detach from the extracellular matrix (ECM). Cancer cells can characteristically gain anchorage independence and survive matrix detachment. Anoikis is averted during Epithelial to Mesenchymal Transition (EMT), a process in which cells express more mesenchymal proteins, promoting their survival. Expression of EMT markers is directly linked to metastatic potential, subsequent disease progression, and poor prognosis. Another family of proteins linked to poor clinical outcome are the small heat shock proteins (sHsps), namely Hsp27 and αB-crystallin, which confer resistance to apoptosis. Investigating how epithelial-derived cancer cells traverse EMT is essential to gain a better understanding of this clinical problem. Although the roles of αB-crystallin and Hsp27 in EMT and metastasis have been investigated in breast and colorectal cancer, their roles in ovarian cancer and anoikis remains to be elucidated. Our laboratory has shown that Hsp27 and αB-crystallin are highly overexpressed in a cisplatin-resistant ovarian cancer cell line (OVCAR8R) as compared to its cisplatin-sensitive syngeneic counterpart (OVCAR8); these cells also express vimentin, a common EMT marker. Therefore, the goal of this study is to characterize the molecular and morphological traits of each cell line when grown detached from a matrix using a model of anoikis. To date, I have successfully generated spheroids via suspended cell culture where spheroids of the cisplatin resistant line are markedly larger than those of the matched chemosensitive cell line. Furthermore, activation of the MAPK/ ERK pathway was abrogated in both OVCAR8 and OVCAR8R cells when cells grow into spheroids as compared to a monolayer, concurrent with upregulation of the mesenchymal protein N-cadherin. Likewise, OVCAR8 cells which lack expression of Hsp27 and αB-Crystallin in a monolayer upregulate the expression of these proteins when detached. Experiments are underway to further analyze spheroids lacking these sHsps to clarify their part in EMT-mediated chemoresistance and proliferation, including immunoblot, immunocytochemistry, and viability assays. Silencing of Hsp27 and αB-crystallin is proposed to reduce expression of metastatic EMT markers, inhibit anoikis and decrease cell viability in ovarian cancer spheroids as compared to cells that express these proteins. To directly address this, we have isolated cells in which Hsp27, αB-crystallin, or both proteins, were silenced via CRISPR-Cas9. Comparison of αB-crystallin and Hsp27 CRISPR-cas9 knockouts will identify important regulatory proteins linked to expression of these anti-apoptotic proteins in ovarian cancer cells. This work will promote a better understanding of the

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mechanisms by which small heat shock proteins promote chemoresistance and prevent anoikis.

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Abstract 2326

A Cockroach model of Parkinsonism

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The American cockroach *Periplaneta americana* has been a model organism for biochemical and physiological study for almost a century, however, its use does not benefit from the genetic tools found in key model species such as mouse and Drosophila. To facilitate the use of cockroaches as a model system in neuroscience, and to serve as a foundation for functional and translational experimentation, a transcriptome of the cephalic ganglia was generated and annotated. We have detected the presence of several genes within the cockroach brain whose homologs in humans and fruit flies are known to be involved in Parkinson's disease, including Parkin. Parking is an E3-ubiquitin ligase and is an important member of a pathway that regulates the health of mitochondria. Mutations in Parkin and are associated with familial (genetic) Parkinson's disease. Knock-down of Parkin in fruit flies causes Parkinsonism and can even be rescued by genetic and chemical interventions. The cockroach's life span is much longer than that of fruit flies (1–2 years vs 40 days) allowing for longitudinal studies for both genetically and environmentally induced Parkinsonism. Further, this allows for testing of interventions such as FDA approved drugs, testing of lead compounds, and changes in diet that may reverse damage or improve symptoms over a longer period of time. The cockroach is amenable to gene knock-down by RNA interference (RNAi). Our major aim was to inject synthetic double-stranded RNA which is cognate to Parkin into adult cockroaches to induce a Parkin-deficient phenotype. We have confirmed the presence of Parkin by PCR and have generated dsRNA based on the sequences obtained. We assessed defects in locomotion such as ataxia, hypokinesia, and initiation of spontaneous walking and diminished grip strength in dsRNA injected cockroaches. Alongside targeting the Parkin gene with dsRNA we have also targeted additional parkinsonism gene homologs such as the D2-like dopamine receptor. The degree of gene knock-down will be investigated using qPCR. The presence of homologs whose mutations are known to be critical in human nervous system diseases may allow for adaptation from Drosophila and mouse models to the cockroach. This approach opens new avenues for experimentation into disease processes and pharmacology over a much longer timeframe than currently available in flies.

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Abstract 2341

Phenotypic and quality assessments of continuously expanded human bone marrow-derived mesenchymal stem cells from multiple donors

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I Kade Karisma Ardana, Ghazaleh Salmanian, Rebekah Samsonraj

Cellular therapy is a rapidly growing field as evidenced by success in preclinical and clinical studies. Of particular relevance are mesenchymal stem cells that have the ability to self-renew, differentiate down multiple lineages, and secrete bioactive factors that render them ideal candidates for tissue repair and regeneration. However, cellular heterogeneity between donors, and intra-donor heterogeneity with repeated subculturing are major bottlenecks in translation to successful cellular therapies. It is not yet known how routine *in vitro* subculturing can impact overall cellular health and functional integrity of the cells. We sought to identify phenotypic differences based on cumulative growth, osteogenic and adipogenic differentiation capacity, secretion of immunomodulatory factors, metabolic parameters, and senescence marker expression on both actively proliferating cells (young MSCs, passages 3–6) and steadily senescing cells (old MSCS, passages 12–18) upon replicative expansion. Our results demonstrate significant differences in osteogenic and adipogenic differentiation capacity of these donors supported by both histological staining and real-time polymerase chain reaction (PCR)-based gene expression studies. Old MSCs exhibit increased senescence-associated beta-galactosidase activity, accompanied by altered immunomodulation capacity as assessed with indoleamine 2,3 dioxygenase secretion and immunosuppressive properties in co-cultures with T cells. We also correlate metabolic marker expression with total time in culture and passage frequency. Together, our results on phenotypic and functional assessments on MSCs subjected to extensive culturing add new knowledge to the field of stem cell biology holding significance in development and optimization of MSC-based cellular therapies for treating degenerative and immune-related disorders.

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Abstract 2342

Investigating the Role of NHE1 in the Development and Progression of Idiopathic Pulmonary Fibrosis

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The chronic lung disorder idiopathic pulmonary fibrosis is a characterized by the thickening, stiffening, and scaring of tissue within the lungs. Individuals impacted by this disease develop shortness of breath and the progressive reduction of respiratory function. The developing fibrosis ultimately results in regions of hypoxia within the tissue, a microenvironment which is closely associated with the upregulation of the sodium-hydrogen exchanger isoform 1 (NHE1). NHE1 regulates a range of cellular functions including cell progression through the cell cycle, reorganization of the actin cytoskeleton and enhancement of cellular motility. When NHE1 is hyperactivated it supports increased cell proliferation and migration, indicating that it may play a vital role in the behavior of fibroblasts in the development of idiopathic pulmonary fibrosis. Our hypothesis is that the inhibition of NHE1 activity would decrease cell progression towards a fibrotic phenotype in lung fibroblasts. To test this hypothesis, we compared differences in cellular behavior in two cell lines to evaluate the role of NHE1 in these processes. LL29 cells are human fibroblasts derived from a patient diagnosed with idiopathic pulmonary fibrosis and PSN cells are Chinses hamster lung fibroblasts expressing only human NHE1. To stimulate changes in cellular function, transforming growth factor beta 1 (TGF-b1), lysophosphatidic acid (LPA), endothelin-1 and serotonin (5-HT) were evaluated for their ability to alter cell proliferation, stress fiber formation, and myofibroblast differentiation. TGF-b1 is a cytokine that is a growth factor implicated in the pathogenesis of pulmonary fibrosis and is involved in differentiation, proliferation, and matrix production of fibroblasts. Serotonin (5-HT) and Endothelin-1 are signaling molecules that bind G-protein coupled receptors supporting the increase in TGF-b1 and its effects in cells. These hormones are involved in wound healing and stimulating inflammation. LPA is a bioactive lipid signaling molecule that plays a role in mediating inflammation and activating TGF-b1 signaling. All experiments were performed in the presence and absence of Ethylisopropylamiloride (EIPA), a potent inhibitor of NHE1, to evaluate the role of NHE1 in fibrotic transformation. In PSN cells, stimulation with TGF-b1 or endothelin-1 increased stress fiber formation 2.19- and 2.45-fold respectively. This stimulation was absent in when cells treated with 10 μM EIPA. In LL29 cells, all four agonists stimulated an increase in cellular proliferation and was abrogated in the present of EIPA. These data indicate a role for NHE1 in the regulation of stress fiber formation and cellular proliferation in idiopathic pulmonary fibrosis.

Leuken Family Foundation.

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ASBMB

Abstract 2346

Investigating the Regulation of the Na+–H+ Exchanger Isoform 1 (NHE1) by Palmitoylation and Phosphorylation

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Mark Wallert

The Sodium Hydrogen Exchanger Isoform I (NHE1) is a 12-pass transmembrane transport protein that exchanges one extracellular Na+ for one intracellular H+ with the primary function of intracellular pH (pHi) regulation. This regulation is essential for the control of cellular proliferation and migration. NHE1 has a 315 amino acid cytoplasmic tail that functions as a regulatory domain. This regulation is extremely complex and includes protein-protein interactions, lipid binding regions, and locations for posttranslational modification, including phosphorylation. It was recently confirmed that NHE1 is palmitoylated in Chinese hamster lung fibroblasts expressing human NHE1 and also in a range of rat tissues. Palmitoylation is a reversible lipid modification, regulating proteins in a dynamic fashion through the addition of the 16-carbon fatty acid (palmitate) to Cys residues through a thioester linkage. This addition is catalyzed by a family of enzymes referred to as palmitoyl acyltransferases (PATs). Palmitoylation is inhibited by 2-bromopalmitate (2-BP), a wide-spectrum PAT inhibitor. NHE1 has five cytoplasmic cysteine residues, and it is currently unknown how many of these residues are palmitoylated. The aim of this work is to investigate the interplay between palmitoylation and phosphorylation in the regulation of NHE1's function and to determine the palmitoylation status of the five cytoplasmic cysteine residues of NHE1. To investigate the interplay between palmitoylation and phosphorylation, we first evaluated the effect of kinase inhibitors on cell proliferation in PSN cells. We utilized inhibitors from the MAPK signaling pathway, PD98059 (MEK) and BI-D1870 (RSK), as well as a non-MAPK inhibitor, Y27632 (Rock), separately and in combination with 2-BP. Through these experiments we identified that BI-D1870 showed combined inhibition on cell proliferation in the presence of 2-BP, displaying a synergistic effect, but the combination of Y27632 and 2-BP did not enhance the level of inhibition on cell proliferation over the use of 2-BP alone. Then, in an effort to identify the palmitoylation status of NHE1, we utilized CRISPR/Cas9 to create NHE1 knockouts in HEK293, A-549, and H1299 cell lines. We will verify NHE1 knock-out by Western blot, qPCR, and Sanger sequencing. This will be the first step in generating Cys to Ala NHE1 knock-in mutants to identify which Cys sites are palmitoylated in NHE1.

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Abstract 2347

Genetic interaction between Ste20 and disease-related mutants of Fig4 in yeast

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Fig4 is a PI3,5P2 5-phosphatase conserved in eukaryotes from yeast to humans. PI3,5P2 levels change in response to both internal and external cellular conditions which regulate many critical cellular processes including endo-lysosomal trafficking, ion channel gating, and TORC1 function. Fig4 is known to modulate levels of PI3,5P2 through direct association with the Fab1-Vac14-Fig4 complex. Mutations in Fig4 have been implicated in multiple human neurodegenerative diseases and altered regulation of PI3,5P2 is believed to underlie Fig4-related disease states. We have recently found that Fig4 disease-related mutations that impair its association with Fab1 and Vac14, promote a growth advantage under specific stress conditions. This growth advantage requires the presence of Fig4, but is conferred independent of Fig4 catalytic function and independent of Vac14. We hypothesize that Fig4 may promote this growth advantage through association with an unrecognized cellular pathway. We have found that the p21-activated protein kinase Ste20 is required for this Fig4-dependent growth advantage, but not for other Fig4-dependent functions. To better understand how Fig4 may influence cell physiology through this unknown pathway, we set out to identify different conditions in which these Fig4 mutants alter yeast phenotypes. Our preliminary data indicate that, in contrast to both wild-type cells and a Fig4 knockout strain, cells expressing Fig4 mutants that are impaired in association with Fab1 and Vac14 can bypass Ste20-dependent glucose-induced cell death. These findings suggest that Fig4 can influence cell fate beyond regulation of PI3,5P2, possibly through a Ste20-related pathway.

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Abstract 2364

A Novel Role for a Histidine Kinase in Bacterial Cell Division

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Streptomyces coelicolor is a Gram-positive filamentous soil-dwelling bacterium. The genus Streptomyces is utilized to produce over two-thirds of the commercially available antibiotics, and its growth is similar to that of fungi. *S. coelicolor* grows using a mycelium-like structure which produces aerial hyphae above the media surface for sporulation. During cell division of these aerial filaments, evenly-spaced crosswalls are developed using division genes, including ftsZ and ftsQ. These genes are essential for growth in common bacteria. However, if these genes are silenced or deleted in Streptomyces, these bacteria will retain their ability for growth. When ftsZ or ftsQ are deleted in Streptomyces, it causes a loss of septum formation in the aerial hyphae, and therefore a loss in spore formation, which can be visualized under the microscope. Research performed in this project has contributed to the discovery and characterization of three new ftsQ-null suppressor strains using visual phenotyping and bioinformatics. These strains were demonstrated to partially compensate for the loss of division in the ftsQ-null mutant. Using whole genome sequencing, it was discovered that all three strains contained a mutation within the same gene, sqnA (suppressor of ftsQ-null). Bioinformatic databases were used to determine that this gene encodes a histidine kinase and is located next to a gene encoding a response regulator. These two genes encode proteins that potentially function together as a two-component regulatory system, which has previously been implicated to play a role in bacterial stress response. Bioinformatic analyses have also been extended to search for the presence of homologues in other bacteria. The current results have demonstrated that there are significant similarities between a wide variety of bacteria. *S. coelicolor* deletion strains for these genes of interest are being constructed using the Lambda REDIRECT recombinase system in both the wild type and ftsQ-null mutant background. Once these strains are successfully constructed, they will be compared phenotypically on plates with different media types and by using phase-contrast microscopy. Novel information produced from this study will further elucidate the cell division process by potentially identifying a new role in division for these genes of interest.

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Abstract 2368

The metabolic fate of exogenous arachidonic acid in the context of ferroptosis

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Ferroptosis is a chemically distinct form of cell death that is implicated in many diseases, including cancer, ischemia, and cirrhosis. It is known that lipid peroxidation drives ferroptosis and that exogenously added polyunsaturated fatty acids (PUFAs) can potentiate ferroptosis. However, the exact lipid species and the molecular events from lipid peroxidation to cell death remain unclear. This study aims to elucidate the metabolic fate of deuterium-labeled arachidonic acid in an *in vitro* ferroptosis model using mass spectrometry-based lipidomics. Briefly, HT-1080 cells were treated with equimolar of d5- and d11-labeled arachidonic acid (dAA) in the presence and absence of a known ferroptosis inducer RSL3 at a non-lethal dose. We first validated the occurrence of ferroptosis in the dAA +RSL3 group by staining lipid peroxides with C11-BODIPY followed by flow cytometry. Lipidomic analysis was carried out using an established HILIC-ion mobility-mass spectrometry-based method, and dAA-containing lipids were selected based on the presence of two m/z values separated by 6 Dalton and with similar intensity. The dAA-containing lipids were then identified using a home-built Python package, Lipydomics, and quantified relative to known internal standards. We found that the dAA was distributed across several lipid classes, with the highest quantity observed in triglycerides (TG, 33%), phosphatidylglycerols (PG, 24%), phosphatidylinositols (PI, 18%), and phosphatidylethanolamines (PE, 15%). In total, 80 deuterated lipids were identified with intensities significantly different between treatment and control groups. RSL3 treatment did not change the overall landscape of dAA-containing lipids, but most identified deuterated lipids, especially those from the PE and PI classes, were lower in the cotreated group than in dAA only. For example, the intensity of PE 40 : 4 is on average 45% lower in the dAA+RSL3 group than in the dAA-only group. +14, +16, and +32 oxidation products were identified for dAA-containing PA, PC, and PE lipids in both dAA and dAA + RSL3 groups, but the ratios of oxidation product/parent lipid are higher in the cotreated cells by up to 47% in PE species. This indicates higher levels of oxidation in cotreated cells, and perhaps a preference for PE oxidation. These results provide the basis for understanding the contribution of each AA-containing lipid class to the execution of ferroptosis *in vitro*.

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ASBMB

Abstract 2371

Preferential interactions of A-kinase anchoring proteins with different protein kinase A subtypes

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A-kinase anchoring proteins (AKAPs) sustain compartmentalization of cyclic AMP signaling cascades within a cell. Discrete compartmentalization at distinct subcellular locations is achieved through selective interaction with different AKAPs. This proceeds through protein-protein interactions maintained by AKAP amphipathic helices on the surface of the anchoring protein that interface with docking and dimerization (d/d) domain proteins. D/D domains were first identified regulatory subunits (type I & II) of protein kinase A (PKA), hence the analogous classification. These substructures consist of a bundle of two parallel and two antiparallel alpha helices. Type I d/d domains contain the same motif plus a short perpendicular helical turn which provides additional contact points creating additional AKAP specificity. Bioinformatic and genomic screening has revealed proteins with similar d/d domains to both type I and type II PKA in cilia (R1D2 and R2D2 proteins, respectively). This study interrogates the link between the AKAP binding preference for the d/d classes, and function in cellular and *in vitro* contexts. To assess *in vivo* AKAP-PKA interactions, tagged AKAP proteins were expressed in HEK293T. Immunoprecipitations identified endogenous binding partners. Cellular analyses by TIRF-FRAP (total internal reflected fluorescence – fluorescence recovery after photobleaching) microscopy were utilized to measure in situ interactions between wild-type and helix mutant AKAPs, and their anchored proteins. Complimentary studies measure the kinetics of the AKAP-d/d protein binding using Octet biolayer interferometry and *in vitro* GST fusion pulldowns. These experiments have defined a hierarchy of binding affinities for different AKAPs and strongly suggest that RII/AKAP interactions are of higher affinity that RI/AKAP interactions. Our studies further suggest that there are few if any dual function AKAPs that show no PKA subtype binding preference inside cells.

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Abstract 2374

MAP Kinase Phosphatases as Nodal Signal Regulators in Health and Disease

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Anton Bennett

The dual-specificity phosphatases (DUSPs) that are responsible for the inactivation of the mitogen-activated protein kinases (MAPKs) by direct dephosphorylation are designated as the MAPK phosphatases (MKPs). The MAPKs are a group of serine/threonine kinases that play important roles in a multitude of cellular processes such as cell proliferation, cell death, and differentiation. Due to their involvement in key cellular functions, aberrant signaling of the MAPKs are associated with a wide range of human diseases. Therefore, the regulatory mechanisms of the MKPs and their impact on the MAPKs is vital for maintaining cellular and organismal homeostasis. We will discuss our recent work on the role of the MKPs in physiological cell signaling and their pathophysiological involvement in diseases such as metabolic and cardiovascular disease. The MKPs have been considered "undruggable." Thus, we have sought to identify new strategies in which to inhibit the MKPs. We will discuss our efforts for targeting the MKPs as potential therapeutics for the treatment of human disease.

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Abstract 2380

Topical formulation containing 3 botanical extracts demonstrate BOTOX-like activity both *in vitro* & *in vivo*

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Geetha Kalahasti, Shona Burkes-Henderson, David Gan, Lucy Gildea

Wrinkles first appear in the lines of facial expression. Dynamic wrinkles, also known as expression lines, are caused by repeated contraction of facial muscles that are attached to the overlying skin. BOTOX is an effective treatment to minimize the appearance of these wrinkles, however many individuals are hesitant to undergo needle injections. Facial muscle contractions are the result of a complex multistep signaling sequence between the nerves and muscle fibers. Calcium is a key regulator of muscle fiber contraction and relaxation. To initiate muscle contraction, nerve cells release factors that activate acetylcholine receptors on the muscle cells. Once stimulated, calcium channels flood the muscle cell with calcium causing actin and myosin proteins to re-organize the sarcomeres and contract the entire muscle fiber. Once the calcium levels reach a threshold, the calcium is pumped out causing actin and myosin resume to their basal state, relaxing the muscle fiber. This constant cycling of calcium levels causes muscles to contract and relax. Here, we identified 3 botanical extracts that were shown to reduce influx of Ca+ and inhibiting myotube contraction of myotubes when stimulated *in-vitro*. A cosmetic formulation featuring these extracts was designed to target the frontalis and procerus facial muscle groups. A proprietary patent-pending clinical methodology was used to evaluate the effectiveness of the cosmetic formulation compared to 20U of BOTOX®. Clinical grading and electromyography (EMG) evaluations occurred at baseline, and after 4 and 8 weeks of product usage. The cosmetic formulation demonstrated significant reduction in the intensity of procerus muscle contraction by 28% at 8 weeks and was shown to be 76% as effective as the standard dosage of BOTOX®. Clinical evaluations also showed significant visual improvement in forehead, glabellar and crow's feet lines and wrinkles after 4 and 8 weeks of use. Overall, this cosmetic formulation was shown to improve the appearance of lines and wrinkles on the forehead and provide an effective alternative to BOTOX injections.

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Abstract 2381

Dependence of Cell Migration on ERK cysteine oxidation

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Extracellular signal-regulated kinase (ERK) is vital in many signaling pathways such as proliferation, differentiation, migration, and even apoptosis. Modulations to the ERK pathway can lead to a variety of diseases. Out previous work has shown that ERK is able to undergo signal-dependent oxidation, and our *in vitro* work suggests that C159 oxidation specifically can alter ERK protein-protein interactions and substrate selectivity. The overall goal of this work is to investigate how oxidation of C159 regulates migration pathways in metastasizing breast cancer cells. To this end, we have used genetically-encodable fluorescent biosensors to monitor ERK activity dynamics at distinct locations in migrating cells. Additionally, in order to determine the role of C159 oxidation in migrating cells, we are developing stable cell lines with inducible WT or C159S co-expressing inducible shRNA to knockdown endogenous ERK. We report our preliminary findings of the role of ERK oxidation on cell migration.

This work was supported by MWRI-Erie and Penn State Behrend joint seed grant.

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ASBMB

Abstract 2382

Investigating alternative treatments for EGFR-driven cancers

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Jeremiah Keyes

Epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase that, under normal functioning, undergoes conformational changes due to phosphorylation and participates in the cells' proliferation and maintenance. Overexpression of this protein is commonly observed in patients with non-small cell lung cancer, a disease responsible for a high percentage of cancer-related deaths in the United States. EGFR is also a protein that is successfully used as a target for cancer drug treatments. Recently, evidence has suggested herbal treatment options may be a successful alternative chemotherapy, radiation, and other common cancer treatments. However, there is little molecular understanding of herbal therapies and limited studies on specific cancer mutations in response to the same treatments. This study aims to investigate cell proliferation and migration behavior within WT and L858R EGFR in response to proven herbal medical treatments as a potential treatment route.

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Abstract 2392

Evaluating Paclitaxel and Na+-H+ Exchanger Isoform 1 (NHE1) Inhibition in Ovarian Cancer

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Erin Becker, Mark Wallert

Every year in the United States there are over 22 500 newly diagnosed cases of ovarian cancer that lead to over 14,000 deaths. This means 1 in 70 women will develop Ovarian cancer in their lifetime. Ovarian cancer is the fourth leading cause of cancerous death among women. A prominent challenge with this disease is drug resistance and disease recurrence resulting from the cancer being commonly diagnosed in an advanced stage. It has been proven that increased activation of the Na+−H + exchanger isoform 1 (NHE1) regulates cell proliferation and intracellular pH in cancer cells from ovarian cancer patients. NHE1 is a transmembrane protein that is comprised of 815 amino acids and is phosphorylated by numerous kinases that influence protein function. NHE1 hyperactivity leads to an intracellular pH inversion which creates an optimal environment for cancer cell proliferation and invasion. To try and combat this, it is hypothesized that combining the secondary chemotherapeutic paclitaxel with inhibitors that directly inhibit NHE1 (EIPA) and inhibitors that inhibit the kinases that activate NHE1 (BI-D1870 and MK-2206) will have a synergistic relationship in slowing the progression of ovarian cancer. Paclitaxel is a microtubule stabilizer that arrests cells in G2/M phase of the cell cycle by hyper-stabilizing tubule formation. This hyper-stabilization induces apoptosis. The direct inhibitor of NHE1 used in these experiments is ethylisopropylamiloride (EIPA). The inhibitors that inhibit kinases that activate NHE1 in these experiments are BI-D1870 which inhibits Rsk and MK-2206 which inhibits Akt. Two ovarian adenocarcinoma cell lines, COAV-3 and SKOV-3, were used to assess the relationship between paclitaxel and NHE1 inhibitors using both XXT proliferation assays and a tumor spheroid assay. Both assays were run with paclitaxel alone and then with both paclitaxel and one of the inhibitors. Initially, IC50 values were determined for paclitaxel in both cell lines based upon data from the XTT assays. It was determined that pairing paclitaxel with EIPA results in an 81.32% reduction in IC50 value in the SKOV-3 cell line and a 43.36% reduction in the CAOV-3 cell line. Paclitaxel in combination with BI-D1870 resulted in a 51.37% reduction in SKOV-3 cells and 64.50% in CAOV-3 cells. The combination of paclitaxel with BI-D1870 and EIPA resulted in an 86.90% reduction value in SKOV-3 cells and 98.89% in the CAOV-3 cell line. Paclitaxel and MK-2206 resulted in a 72.93% reduction in CAOV-3 cells and 99.99% in SKOV-3 cells. The tumor spheroid assays were conducted to evaluate whether a similar pattern of efficacy occurred in 3D culture. Preliminary spheroid data indicates that the inhibition with paclitaxel and EIPA were consistent in the CAOV-3 cell line but not in the SKOV-3 cell line. Eventually this relationship will be tested with NHE1 knockout cell lines as well. This will validate the role NHE1

plays in cancer progression. These data indicates that pairing paclitaxel with an inhibitor or NHE1 or an inhibitor of NHE1 kinases may improve the efficacy of ovarian cancer treatments.

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Abstract 2397

AKAP2 as a breast cancer signaling scaffold

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Frank Smith, John Scott

Compartmentalization of protein kinases ensures spatial specificity in intracellular signaling. One way that cells localize signaling cascades is through A-kinase anchoring proteins (AKAPs). This family of multiprotein scaffolds position Protein Kinase A (PKA) and other signaling enzymes at distinct subcellular locations in proximity to substrates. Recent evidence suggests that pathological changes in anchored signaling events are linked to aberrant AKAP expression in cancers. In breast cancer, we have found that excessive expression of the cytoskeletal anchoring protein AKAP2 is positively correlated with migratory and invasive cancer cell lines. AKAP2 expression is also positively correlated with highly invasive triple negative breast cancer signaling pathway expression. Conversely, AKAP2 expression is negatively correlated with a less invasive phenotype in epithelial breast cancer lines. We postulate that AKAP2 anchors signaling enzymes at the cytoskeleton that are involved in cell motility. Accordingly, our experiments show that shRNA depletion of AKAP2 increases cell migration, suggesting that AKAP2-binding partners modulate cell motility. Through co-immunoprecipitation studies, we show that the mitotic kinase, polo-like kinase 1 (Plk1), is an AKAP2 binding partner in triple negative breast cancer cells. This suggests that AKAP2-mediated localization of Plk1 may play a role in proliferative activity. Co-immunoprecipitation/LC-MS screening with triple negative breast cancer cells revealed multiple AKAP2-binding partners which are involved in cancer cell invasion and cell cycle regulation. The objective of these studies is to characterize the role that AKAP2 anchoring has in pathological breast cancer signaling. Through this work, we can gain better understanding of the role of AKAP2 signaling complexes in triple negative breast cancer to develop better treatments.

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Abstract 2398

CCN proteins as Downstream Components of Lysophosphatidic Acid Signaling in Prostate Cancer Cells

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Brianna Knode, Kathryn Meier

Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are bioactive phospholipids, known for their multitude of effects in various cancers. Both LPA and S1P elicit responses by activating their cognate G-protein coupled receptors (GPCRs). CCN1 and CCN2 are matricellular proteins that are induced by LPA and S1P in various disease states including cancers. Our group has previously shown that 18:1-LPA increases proliferation and migration in human prostate cancer cell lines through the LPA receptor LPAR1. Expression of CCN1 is induced 2–6 hours after LPA addition. However, the roles of CCN proteins in GPCR-mediated responses are still unclear and are of growing interest. We hypothesize that LPA-induced CCNs play a key role in mitogenic signaling and cell adhesion in prostate cancer cells. For signal transduction assays, whole-cell extracts and extracellular matrix from PC-3 cells treated ±10 μM 18:1-LPA or S1P were subjected to immunoblotting. For immunofluorescence experiments, cells grown on cover slips were treated ±10 μM LPA, fixed, and stained for CCN1. The results show that CCN1 and CCN2 protein levels were increased after 2–4 hours by S1P or LPA. LPA-induced Erk MAPK activation was biphasic, with the later phase occurring after CCN1 induction. For adhesion assays, PC-3 cells were serum starved for 24 hours and then plated on fibronectin-coated plates ± 10 μM LPA for 2 hours. Adherent cells were stained and quantified. PC-3 cells treated with LPA exhibited increased adherence concomitantly with increased CCN1. For proteomics experiments, cells were treated with interfering RNA for CCN1 for 48 hours, and then incubated ± 10 μM LPA for 3 hours. The global proteome of whole-cell extracts was acquired using the data independent acquisition (DIA) method. The results of the proteomics experiments revealed changes in the expression of multiple proteins after LPA addition; this profile was altered after CCN1 knockdown. Our study emphasizes that CCN proteins are key components of LPA-induced responses, making them viable therapeutic targets to be further explored.

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Abstract 2413

Is testosterone the male sex hormone responsible for Increased male mortality in Melanoma? *In-Vitro* studies on a human Melanoma (1205Lu) Cell Model

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Our previous study with the BLM human melanoma cell line indicated that testosterone (T) was not able to suppress interleukin-8 (IL-8) secretion and melanoma cell growth like progesterone, suggesting the inefficiency of the T in controlling IL-8 secretion and melanoma cell growth as the probable causes for increased male mortality in melanoma. In order to confirm the above finding, the study was repeated with another human melanoma (1205Lu) cell line. Our aims were to check the efficiency of T in decreasing melanoma cell growth and IL-8 secretion 1) by indirect induction of IL-8 2) by direct addition of IL-8 and 3) by suppression of endogenous IL-8 secretion followed by direct addition of IL-8 to the cells. In order to address these questions, it was decided 1) to induce endogenous IL-8 in melanoma cells by pre-incubating with endothelin (50 ng/ml) and then adding steroids (androstenedione AD, testosterone T, progesterone P) 2) to add IL-8 at two concentrations (100 pm and 10 ng) directly to the cells and then add steroids and 3) finally suppress endogenous IL-8 by pretreating the cells with curcumin (25 μM) and then add IL-8 along with steroids. It was noticed that T was not able to suppress cell growth in the cells pretreated with endothelin to induce endogenous IL-8. Similarly, when IL-8 was directly added along with T, the cell growth was slightly higher in T+IL-8 cells compared to plain T-treated cells. The addition of T to curcumin-pretreated cells significantly decreased cell growth. But, when IL-8 was added, the cell growth was partially restored suggesting the inefficiency of T to sustain the inhibition of cell growth in the presence of IL-8. Elisarray of the supernatants and the quantitation of IL-8 by Elisa also pointed to the inefficiency of T to suppress IL-8 secretion.

Conclusion: These experiments endorsed our previous findings with BLM cells and the conclusion that in males 2 reasons 1) a deficiency of progesterone and 2) inefficiency of T to suppress endogenous IL-8 could possibly result in an increased IL-8 level. This increased IL-8 level in males could lead to increased cell growth and metastasis in melanoma leading to death (IL-8 had already been shown to stimulate melanoma cell growth and metastasis by us and others). So, a situation arising out of male reproductive endocrine physiology could probably be responsible for increased male mortality in melanoma.

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Abstract 2419

Elucidating the Molecular Function of the Autism-Associated Kinase NUAK1 in Neurodevelopment and Disease

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Smita Yadav

The serine-threonine kinase NUAK1 is highly expressed in the developing brain, where it regulates terminal axon branching by promoting presynaptic mitochondrial capture through an unknown mechanism. Several de novo mutations in NUAK1, which encodes the AMPK-related Nua kinase 1 (NUAK1), have been reported to be associated with autism spectrum disorder (ASD). The role of NUAK1 in this process appears to be dependent on NUAK1 kinase activity, but the direct neuronal phosphorylation substrates of NUAK1 have not been characterized. Further, the impact of ASD-associated mutations on the molecular function of NUAK1 in neuronal development is unknown. To identify the direct substrates of NUAK1 in the brain, I have taken a chemical-genetic approach by genetically engineering the gatekeeper residue M132 of NUAK1, allowing it to utilize bulkier ATPgS analogs instead of ATP. Through combinatorial assessment of different bulky ATP analogs and NUAK1 analog sensitive mutants, I found that M132G showed a 600% increase in utilization of Phenyl-ATPgS over the wildtype NUAK1. I further optimized *in vitro* substrate labeling by NUAK1 in mouse brain lysate, which will be used to identify protein substrates of NUAK1 using mass spectrometry. To investigate how ASD-associated mutations in NUAK1 affect its molecular function, I have utilized *in vitro* kinase assays and confocal microscopy to determine that each mutation has a unique impact on NUAK1 kinase activity and subcellular localization. I next plan to characterize how these mutations affect axonal development *in vitro* in cultured rat neurons. This research will elucidate the mechanism of the understudied kinase NUAK1 in axonal development, as well as define the hitherto unknown downstream substrates of NUAK1 important for axonal development.

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Abstract 2421

LPA and TGF-β coordination of fibrosis requires NHE1

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Meera Iyer, Joseph Provost

Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease characterized by scarring and stiffening of the lung tissue. As a result, patients with IPF experience difficulty breathing and reduced oxygen uptake. Under normal conditions, fibroblasts secrete collagen proteins that compose the structural framework of tissues. In IPF, profibrotic agonists activate fibroblasts causing them to lay down excessive collagen and extracellular matrix. Transforming growth factor beta (TGF-β) is a profibrotic cytokine involved in fibroblast function that is upregulated in fibrosis. Lysophosphatidic acid (LPA) is a phospholipid derivative that enhances fibrosis by recruiting myofibroblasts leading to an accumulation of extracellular matrix (ECM). Many of the fibroblast behaviors observed in IPF, increased proliferation, stress fiber formation, increased motility have been observed in non-small cell lung cancer. Our lab has shown that these functions require the activity of the sodium hydrogen exchanger isoform 1 (NHE1). Therefore we hypothesized that NHE1 would play a supportive role in the progression of IPF. This study focuses on the relationship between these two cytokines and their involvement in fibroblast differentiation and the progression of lung fibrosis. We found that 10 μM LPA induced lung fibroblasts to secrete active TGF-β 1.8 fold over basel TGF-β production. Addition of a specific NHE1 inhibitor, Ethyl Isopropyl Amiloride (EIPA) blocked the secretion of active TGF-β to background levels indicating that LPA activation of TGF-β requires the exchanger. Interestingly, TGF-β increased IL-6 secretion in an NHE1 dependent manner but not LPA, indicating a different mechanism of action. To examine the role of NHE1 on cell proliferation, Chinese hamster lung cells (CCL39) were seeded into 96-well plates with different pro-fibrotic agonists (TGF-β and LPA) in the presence and absence of EIPA. The data on both fibroblasts and myofibroblasts will provide further information on the feedback loop of LPA on TGF-β and a possible role of NHE1 in this signaling pathway. This draws a correlation between NHE1 and fibroblast differentiation that identify a novel modality to treat fibrosis.

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Abstract 2436

Fluorescence Resonance Energy Transfer (FRET) Spatiotemporal Mapping of Atypical P38 Reveals an Endosomal and Cytosolic Spatial Bias

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Jenifer Okalova, Jeremy Burton

Mitogen-activated protein kinase (MAPK) p38 is a central regulator of intracellular signaling, driving physiological and pathological pathways. With over 150 downstream targets, it is predicted that spatial positioning and the availability of cofactors and substrates determine kinase signaling specificity. The subcellular localization of classical mitogen-activated kinase kinase 3/6 (MKK3/6) dependent p38 is highly dynamic to facilitate the selective activation of spatially restricted substrates displaying rapid nuclear translocation. In addition to classical MKK3/6-dependent p38 activation, the adaptor protein TAB1 can selectively bind to p38, inducing p38 autophosphorylation in a pathway termed atypical p38 activation. Recent studies have linked atypical p38 activity to a wide range of pathological signaling responses, including vascular inflammation and ischemic damage. However, the spatiotemporal profile of atypical p38 inflammatory signaling has not been studied and would provide valuable insight into how atypical p38-driven pathological responses. To address this critical gap, we developed genetically encoded fluorescence resonance energy transfer (FRET) biosensors to track p38 activity with subcellular resolution. Through comparative analysis of plasma membrane, cytosolic, nuclear, and endosomal compartments, we established a characteristic profile of nuclear bias for MKK3/6-dependent p38 activation. Conversely, atypical p38 activation via thrombin-mediated G-protein coupled receptor (GPCR) protease-activated receptor 1 (PAR1) activity led to the sequestration of p38 at the endosome and cytosol, limiting nuclear translocation, a profile conserved for additional inflammatory GPCRs, including prostaglandin E2 activation of EP2. Intriguingly, perturbation of GPCR endocytosis with the dynamin inhibitor Dyngo4A led to spatiotemporal switching of atypical signaling, reducing endosomal and cytosolic p38 activation and increasing nuclear activity. Suggesting that a critical and yet unknown endosomal regulator drives the spatial bias of atypical p38 signaling. The data presented provide the first live-cell examination of the spatiotemporal dynamics for p38 activity and provide critical insight into how atypical p38 signaling drives differential signaling responses through spatial sequestration of kinase activity.

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Abstract 2439

Effect of JTT-551 inhibitor on the catalytic activity of wild type and mutant forms of PTP1B

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Ariel Shalonov

Kinases and phosphatases regulate intracellular signal transduction pathways by reversible protein phosphorylation process. Protein Tyrosine Phosphatase 1B, PTP1B, is the most characterized phosphatase in all protein tyrosine phosphatases. It was first identified as a negative regulator of insulin and leptin signaling and later as tumor suppressor and tumor promoter through dephosphorylation of tyrosine residues of proteins in receptor tyrosine kinase signaling pathways. This makes PTP1B an important therapeutic target not only in diabetes and obesity but in human cancers as well. In fact, mutations in PTP1B have been found in various cancers. Here, we characterized different mutant forms of PTP1B and observed the effect of mutations on the catalytic activity of PTP1B. Using artificial substrate para-nitrophenol phosphate, we measured kinetic parameters for the mutant forms of PTP1B and compared to the wild type. We also examined effect of JTT-551, a small molecule inhibitor of PTP1B on the catalytic activity of wild type and mutant forms of PTP1B. Our results showed significant reduction in catalytic activity upon mutations and the inhibitor was not effective in inhibiting mutant forms of PTP1B. This initial characterization will be helpful in deeper understanding of the role of PTP1B and also provide insight for drug design in developing inhibitors for mutant forms of PTP1B.

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Abstract 2440

Detecting interactions between Rho GTPase RhoJ and coronin proteins using split luciferase protein complementation

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Rachel Murphy, Chelsea Samuelson

RhoJ is a Rho family GTPase that contributes to tumor-associated angiogenesis; however, less is known about its fundamental biochemistry and cellular biology. Previously, our lab has found that GTP-loaded RhoJ tends to localize to the plasma membrane, while GDP-loaded RhoJ localizes to intracellular vesicles. In addition, our data suggests that vesicular localization of GDP-loaded RhoJ occurs through a protein/protein interaction. Coronin proteins are a logical partner for this interaction since they selectively bind GDP-loaded Rho-family GTPases and have been shown to have some functional overlap with RhoJ. Direct interactions between full length coronins and RhoJ were tested in a split luciferase protein complementation assay, where cDNA encoding human coronin isoforms were recominantly fused to one fragment of split luciferase and RhoJ to the other. Our initial data indicates that RhoJ has the strongest association with Coronin1C, and with 2A to a lesser extent. Given the importance of RhoJ in tumor-associated angiogenesis, determining its protein/protein interactions will help illuminate its role in this pathological process.

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Abstract 2444

Antibacterial Agent, Triclosan (TCS), Alters the Production of Pro-Inflammatory Cytokine Interleukin 1 beta from Human Immune Cells: The Role of Mitogen-Activated Protein Kinases

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Margaret Whalen

Triclosan (TCS) is an antimicrobial compound widely used in personal hygiene products such as mouthwash and toothpaste; as a result, TCS has been found in human blood, breast milk, and urine. Interleukin-1 beta (IL-1β) is an important pro-inflammatory cytokine produced by lymphocytes, monocytes, and other cells. IL-1β regulates cell growth, tissue repair, and immune function, increased levels in the absence of appropriate stimuli (injury or infection) can lead to chronic inflammation which is been associated with many diseases, including rheumatoid arthritis and certain cancers. Previous studies have shown that TCS stimulated secretion of IL-1β by immune cells and that this TCS-induced increase was dependent on mitogen-activated protein kinases (MAPKs). The current study examines whether this increase in secretion is due to release of already existing stores of IL-1β or if TCS is able to stimulate cellular production (both secreted and intracellular levels) of IL-1β. Additionally the study addresses the role of MAPKs in any TCS-induced increases in production. Human peripheral blood mononuclear cells (PBMCs) were exposed to TCS at concentrations of 0–5 μM. The cellular production of IL-1β was measured at 10 minutes, 30 minutes, 6 hours, and 24 hours. Secreted levels were measured in supernatants from exposed cells using enzyme-linked immunosorbent assay (ELISA) and intracellular levels were measured by Western Blot. Results indicate the production of IL-1β was increased by exposure to one or more concentration of TCS at each length of exposure. The greatest increase in IL-1β production was seen at 6 h, where all TCS exposures caused substantial increases in IL-1 β production. The role of MAPKs (p38 and ERK1/2) in this TCS-induced stimulation of IL-1β production was examined by pretreating PBMCs with a selective inhibitor of p38 (SB202190) and a selective inhibitor (PD98059) of the immediate upstream activator of ERK1/2, MEK, for 1 h followed by a 6 h exposure to 5, 2.5, and 1 μM TCS. IL-1β production was measured as described above. The results showed that both p38 and ERK 1/2 were needed for TCS to induce increased IL-1β production by immune cells. Using RT-PCR, it was shown that the increase in IL-1β production stimulated by TCS was accompanied by increased mRNA for the protein. These results of these studies verified that TCS increases immune cell production of IL-1β and that this increased production is dependent on MAPK pathways. The ability of TCS to increase production indicates that rather than activating a self-limiting process of depleting cells of already existing stores of IL-1β, TCS is able to stimulate a process that has the capacity to provide sustained production of

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IL-1β and thus may lead to chronic inflammation and its pathological consequences.

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Abstract 2447

Serotonin-secreting enteroendocrine cells are key mediators in the colon to cellular stresses via ATF4-ATF3 mediated stress-responsive signaling

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Siyuan Liang, Vivian Zheng, Guillermo Vargas Garcia, Yuan-Xiang Pan, Hong Chen

Objectives: In a previous study, we found that the proportion of enteroendocrine cells (EECs), and one of its products, serotonin, were significantly increased in the colon of a colitis mouse model. In addition, the integrated stress response (ISR) was activated in these EECs. In this study, we aimed to investigate cell-cell communications and identify ligand-receptor interactions regulating stress responses. We also examined the signaling pathways contributing to the outcomes following EEC stress responses. We hypothesize that signal transduction via Amphiregulin (AREG)/Epithelial growth factor receptor (EGFR) is essential in assisting EECs to recognize and respond to stresses, while ATF4-ATF3 Mediated Wnt/β-Catenin Signaling is the primary target following stress responses within EECs.

Methods: CellChat (R package, version 4.0) was used to map cell-cell communications in the colon of the Hnrnp I knockout mouse model of colitis. The murine intestinal secretin cell line (STC-1) was used to confirm mRNA and protein expressions of key regulators in the identified signal transduction pathways because it possesses many features of native intestinal enteroendocrine cells. Amino acid deprivation (-AA) was treated to trigger cellular stress responses and to compare to untreated cells (CON). RT-qPCR technique was used to quantify mRNA expression levels, while western blot and immunofluorescence staining were used to quantify and visualize protein expression levels and locations. All measurements were performed in replicates. Results were analyzed using an unpaired t-test, with P-value <0.05 considered significant.

Results: Gene expression of cellular stress response factors – Atf3 and Atf4, was significantly increased in amino acid-deprived cells compared to CON. Immunofluorescence staining of the proteins suggests that both ATF3 and ATF4 protein expressions were elevated in the nucleus of amino acid-deprived cells, indicating their activations. P21, a stress response signaling target and a marker of cell apoptosis, was also significantly upregulated in -AA cells. Following stress response, amino acid-deprived cells demonstrated activation of Wnt signaling through upregulation of Wnt5a gene expression and nuclear translocation of β-CATENIN. Cox2, a target of Wnt signaling, and mediator of cell proliferation, was also significantly upregulated in amino acid-deprived cells. The phosphorylated p44/42 MAPK (ERK1/2) protein expression was not significantly impacted in amino acid-deprived cells, which suggests that the cellular stress responses were independent of the MAPK pathway. Meanwhile, the production of

the key intestinal neurotransmitter Serotonin (5-HT) from EEC cells is also dysregulated.

Conclusions: The results from the current study strongly indicate that intestinal EECs actively respond to cellular stresses via ATF4-ATF3 Mediated Wnt/β-Catenin Signaling. This may further influence cellular proliferation and apoptosis as it regulates gene expression of key factors in cell cycle regulation and inflammatory responses. Furthermore, although integrated stress responses are often closely related to MAP kinases extracellular signal-regulated kinase (ERK) 1/2, our results showed that the activated signaling following increased stress level in EECs is MAPK-independent. Further investigation will focus on the signal transduction via Amphiregulin (AREG)/ Epithelial growth factor receptor (EGFR), potentially facilitating colonic EECs in responding to stress.

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Abstract 2450

Altered expression of Rho GTPase TCL/RhoJ in HT29 cells grown under different media conditions

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Michael Hamann

TCL/RhoJ is member of the Rho-GTPase family and is found to be preferentially expressed in endothelial cells, where it is thought to selectively promote tumor-associated angiogenesis. This process occurs when hypoxic tumor masses secrete pro-angiogenic factors, such as VEGF, which impact on nearby endothelial cells, causing them to mitose, infiltrate tissues, lumenize, and vascularize hypoxic tumors. The experiments presented here seek to address if TCL expression levels can be impacted by nutritional considerations, thus providing a mechanism to limit its role in tumor-associated angiogenesis. In this study, we examined a selected gene expression profile of HT-29 cells grown in DMEM or RPMI media by qPCR. Specifically, we analyzed transcript level of the genes TCL, Muc2, LGR5, and HGPRT. Analysis of the gene transcription levels indicates an elevated expression of TCL and LGR5 in cells grown in DMEM media, and elevated Muc2 transcript levels in RPMI, normalized to HGPRT expression levels. Additional experiments will begin to identify the component(s) of DMEM or RPMI that may be impacting TCL gene expression. These results suggest that available nutrients and microenvironment around tumors and endothelial cells may play a role in the promotion of tumor-associated angiogenesis.

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Abstract 2459

Elucidating the role of autism risk gene TAOK2 in neurodevelopment and disease

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Smita Yadav

The autism risk gene TAOK2 encodes a serine/threonine kinase important for several aspects of neurodevelopment including axon elongation, dendritic branching, and spine/synapse formation. We have recently discovered that TAOK2 is an endoplasmic reticulum (ER) membrane localized protein kinase and functions to tether the ER to microtubules (MT). Despite its importance in neuronal development and strong disease association, the molecular mechanisms underlying the role of TAOK2 in neurodevelopment and its biological function as an ER-MT tether are not understood. Here, through time lapse confocal microscopy in human induced pluripotent stem cell (hiPSC)-derived neural progenitor cells and neurons, we found that loss of TAOK2 function disrupts ER dynamics. Further, through live imaging and *in vitro* kinase assays, we discovered that autism associated TAOK2 mutations differentially affect its subcellular localization and catalytic activity. Our results suggest that TAOK2 mediates neuronal development through regulation of ER-MT tethering, dysfunction of which may contribute to pathophysiology of ASD.

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Abstract 2500

The Effect of Sildenafil on Atrial Natriuretic Peptide Level in Rats Treated with Monocrotaline

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Mukhallad AlJinabi

Pulmonary hypertension is associated with an increase in pulmonary vascular resistance leading to pulmonary vascular changes, right ventricular hypertrophy, and heart failure. Pulmonary hypertension can be induced in animal models similar to humans where the changes are comparable. Monocrotaline (MCT), an alkaloid derived from a toxic plant, can be used to induce pulmonary hypertension in animals. Sildenafil has been used to treat patients with pulmonary hypertension since it induces smooth muscle relaxation. Atrial natriuretic peptide (ANP) is a hormone secreted naturally from the atria of the heart. An increase in ANP in hypervolemia and hypertension is beneficial since it induces a significant decrease in blood volume, therefore, lowers blood pressure. Studies showed that atrial natriuretic peptide combined with sildenafil reduces pulmonary arterial hypertension significantly. Cardiac hypertrophy due to pulmonary hypertension increases expression of myosin heavy chain β in the right ventricle of the heart. Aims: To explore the ability of sildenafil to alleviate PH induced by Monocrotaline (MCT) in rats and explore the ANP level before and after induction of PH, and the administration of sildenafil.

Methods: Sixty four male Wister rats, were divided randomly into 4 groups: Control A, (7 Rats). Group received MCT only (14 rats), at dose 60 mg /kg body weight subcutaneously. Group C received a single injection of MCT subcutaneously at a dose of 60 mg /kg for 3 weeks and then sildenafil at a dose of 50 mg/kg/day from the beginning of the fourth week to the end of the sixth week (The reversal group) counted 23 rats. Group D received a single injection of MCT subcutaneously at a dose of 60 mg /kg for 3 weeks at the same time sildenafil was given at a dose of 50 mg/kg from day 1 to the end of day 21 (The prevention group) consisting of 20 rats.

Results: The survival rate of the prevention group was the highest compared to other groups. The results revealed that MCT-treated rats and the reversal group showed a significant increase in ANP levels compared to the control one. The prevention group rats showed a significant decrease in ANP levels compared to both the reversal and the MCT-treated group. Fulton index ratio shows a significant increase in MCT treated rats and the reversal group compared to the control group, also it has an increasingly significant level to the reversal group compared to the prevention group. The percent of β-myosin heavy chain (MHC) is decreased significantly in the reversal group compared to both the MCT treated group and the control group. Nitric oxide (NO) levels were increased significantly in the reversal group compared to both the MCT treated group and the control group. Conclusion: Sildenafil

significantly decreases ANP levels in rats treated with MCT, and it also decreases cardiac hypertrophy induced by MCT.

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Abstract 2509

Decoding New Players in Plasmodium Nuclear Biology using Expansion Microscopy

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Benjamin Liffner

Infection by the protozoan parasite *Plasmodium falciparum* causes the most severe form of human malaria. Clinical manifestation of malaria begins with the parasite invasion and proliferation in human red blood cells (RBC). Following the invasion of an RBC, a single-celled parasite replicates into ~20 new daughter parasites through an atypical cell division process named schizogony. Unlike its human host, *P. falciparum* undergoes multiple rounds of asynchronous DNA replication and nuclear division without cytokinesis to form a multi-nucleated parasite, followed by a single round of specialized cytokinesis where individual nuclei and subcellular organelles are partitioned to produce daughter parasites. This distinctive replication mode relies on timely DNA replication, segregation, and nuclear division within an intact nuclear envelope in a shared cytoplasm. To study the molecular processes occurring within an ~1 μm Plasmodium nucleus, we turned to Ultrastructure expansion microscopy (U-ExM), a recently developed sample preparation method for microscopy. U-ExM allowed us first to achieve the resolution needed to visualize intranuclear microtubule dynamics during nuclear multiplication. Moreover, we developed the first Plasmodium nuclear envelope staining enabled by expansion microscopy. We are now successfully applying our microscopy advances to determine the role of assembly/disassembly of microtubules, kinase, and nuclear condensate in DNA segregation and nuclear division. Our laboratory aims to uncover critical processes for the atypical cell division of malaria parasites that remain poorly understood and will likely reveal druggable targets for developing novel antimalarials urgently needed due to the emergence of resistance against all current treatments.

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Abstract 2511

Illuminating the Biochemical Activity Architecture of the Cell

Jin Zhang, *UC San Diego*

The complexity and specificity of cellular processes require spatial microcompartmentation and dynamic modulation of the underlying biochemical activities, such as dynamic phosphorylation and dephosphorylation catalyzed by specific protein kinases and phosphatases, respectively. We hypothesize that cellular biochemical activities are spatially organized into an "activity architecture." We further hypothesize that disease states such as cancer reflect reorganization and restructuring of this activity architecture. In this talk, I will introduce a series of genetically encoded fluorescent biosensors that we have developed to achieve single cell analyses of biochemical activities, and then present a couple of studies where we combine live-cell imaging with targeted perturbations as well as biochemical and functional assays to probe the mechanisms and functions of spatiotemporal regulation of cAMP/PKA and ERK signaling pathways.

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Abstract 2517

The intensities of canonical senescence biomarkers integrate the duration of cell-cycle withdrawal

Sabrina Spencer, *University of Colorado - Boulder*

Sabrina Spencer, Humza Ashraf, Brianna Fernandez

Senescence, a state of permanent cell-cycle withdrawal, is difficult to distinguish from quiescence, a transient state of cell-cycle withdrawal. This difficulty arises because quiescent and senescent cells are defined by overlapping biomarkers, raising the question of whether quiescence and senescence are truly distinct states. To address this, we used single-cell time-lapse imaging to distinguish slow-cycling quiescent cells from bona fide senescent cells after chemotherapy treatment, followed immediately by staining for various senescence biomarkers. We found that the staining intensity of multiple senescence biomarkers is graded rather than binary and primarily reflects the duration of cell-cycle withdrawal, rather than senescence per se. Together, our data suggest that quiescence and senescence are not distinct cellular states but rather fall on a continuum of cell-cycle withdrawal, where the intensities of canonical senescence biomarkers reflect the likelihood of cell-cycle re-entry.

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Abstract 2519

Engineering nature's sensors into scientists' switches

Kevin Gardner, *CUNY Advanced Science Research Center*

Environmental cues regulate many biological processes, coordinating cellular pathways to respond to changing conditions. Such regulation is often initiated by sensory protein domains which expand their chemical repertoire by using small molecule ligands to convert environmentally-triggered changes into altered protein/protein interactions. Using a combination of biophysics, biochemistry and synthetic chemistry, we study the mechanistic controls of such domains for both fundamental understanding and subsequent artificial control. Here I will focus on these principles in LOV (Light-Oxygen-Voltage) type blue light photoreceptors, which convert photochemically-driven covalent changes at their flavin chromophores into structural alterations at the protein level. With examples from a diverse group of naturally-occurring LOV proteins, I will discuss how our integrated approach provides insights into the commonalities and differences in signaling processes among this group. I will close by providing examples of how such information guides subsequent protein engineering for *in vitro* and cellular applications.

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Abstract 2545

Dissecting time and space dynamics within the EGF Receptor signalosome

Claire Martin, *Sinai Health System*

James Knight, Kento Abe, Bhavish Rathod, Cassandra Wong, Brett Larson, Anne-Claude Gingras

Epidermal growth factor receptor (EGFR) is a key regulator of cell proliferation and its inappropriate activity is commonly associated with cancer. Despite intense study over the past 60+ years, a complete understanding of the mechanisms governing EGFR signaling remain unclear. BioID is a powerful mass spectrometry-based footprinting technique used to identify protein-protein associations in an unbiased manner. By integrating BioID with a systems biology approach, we set out to generate a spatiotemporal map of the proteins contributing to EGFR signal regulation. Leveraging the speed of the recently developed miniTurbo BioID enzyme, we monitored EGFR's changing protein neighbourhood at 10 timepoints following EGF-stimulation. By clustering the ~200 high-confidence interactors identified based on their reorganization at EGFR over time, we uncovered distinct patterns of association for proteins at specific plasma membrane microdomains and involved in regulation of EGFR signaling, endocytosis and trafficking. The interdependence and sub-signalosome organization of these partners was further clarified by reciprocal BioID characterizations of EGFR neighbours (15), and by coupling BioID with CRISPR-Cas9 gene editing (>25 targets) or EGFR mutagenesis (6 sites). Our findings suggest a lose scaffold of signaling/endocytic/trafficking proteins exists in resting cells. Upon stimulation, scaffold members become highly interconnected, and signaling and endocytic proteins concentrate together at EGFR. GRB2, an already well-established EGFR signaling adaptor, is essential for node formation. Further, we uncover an EGF- and GRB2-dependent relationship between Y-phosphorylated EGFR and the poorly characterized ubiquitin-binding protein SPATA2, localized at this node. Genetic disruption of SPATA2 enhances EGFR Y-phosphorylation and halts its turnover. SPATA2 perturbations are common in cancer (cBioPortal.org), and ongoing efforts aim to determine how the SPATA2-EGFR relationship might contribute to disease. Altogether, these data provide novel insights into EGFR signaling regulation and demonstrate the proficiency of using miniTurbo BioID and a systems biology approach to investigate protein complex rewiring following cellular stimulations.

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Abstract 2550

Live cells single molecule biochemistry: what can we learn by interrogating molecular interactions in their cellular context?

Xavier Darzacq, *University of California-Berkeley*

Molecular interactions are at the center of cellular regulation processes. There is currently a revolution in live-cell super resolution imaging. I will discuss how our recent data measuring these interactions at the single molecule level and in live cells has changed our understanding of how molecular assemblies are controlled in cells. We will discuss how a balance in between strength and numbers plays a role in controlling interactions and how we are including "on-rates" as an additional regulation mechanism to the off-rates typically studied in biochemical studies. This will be illustrated with examples from our work on chromatin organization and transcription regulation. Using examples from our published and unpublished experiments I will illustrate how molecular interactions in cells are the result of multiple protein domain contributions leading to a complex network of cooperating or competing interactions more prone to regulation than our typical 1:1 domain docking models.

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Abstract 2558

Decoding mechanisms that control PPP specificity

Rebecca Page, *UConn Health*

The large majority of ser/thr dephosphorylation is performed by the PPP family, comprised of just seven families: PP1, PP2A, PP2B/PP3/calcineurin (CN), PP4, PP5, PP6 and PP7. While it has been known for more than two decades that PP1 and CN engage their regulators using short linear motifs (SLiMs), the emerging view is that regulator and substrate engagement via SLiMs is likely conserved throughout the entire PPP family, with SLiMs now identified for PP4 and PP2A-B56. Remarkably, new mechanisms that modulate regulator and substrate binding continue to be discovered. For example, we recently discovered that dynamic charge-charge interactions modulate the affinities of PPP-specific SLiMs for their cognate PPPs. We also discovered that similar dynamic electrostatic interactions can, in some cases, actively direct substrate specificity. Finally, we have also shown that SLiM phosphorylation has opposing effects on PPP binding, leading to differential PPP recruitment to the same substrate in distinct cellular states. Here, we will present new data illustrating some of the diverse and novel mechanisms used by regulators and substrates to engage their cognate PPPs and, in turn, direct PPP holoenzyme formation and activity.

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Abstract 2581

GRPR- a potential role in radio-sensitization

Jycole Bush, *Fort Lewis College*

Michelle Villarreral, Addanki Kumar

Disease relapse and treatment-associated side effects preclude optimum quality of life for prostate cancer (PCa) patients and serve as critical barriers for effective management of the disease. Therefore, there is an urgent need for innovative target-based personalized strategies that can prevent relapse and improve quality of life. Radiation therapy (RT), the most common type of therapy used to treat majority of cancers including prostate damages the cancer cells and even normal cells by depositing energy into the cells which causes the cell death or even genetic damage which also results in cancer cell death. However, over time, prostate cancer cells can develop resistance to radiation, also called radio-resistance, which makes treatment even more difficult. A technique that is being investigated to increase the effects of radiation to the target area and decrease the toxicity to the normal tissue is the use of radio-sensitizing agents. Studies from our laboratory demonstrate potential utility for targeting ribosomal protein S6K (encoded by RPS6KB1) and NAD+ dependent deacetylase Sirtuin 1 (SIRT1) in radio-sensitization. Unpublished and recently published studies show that genetic or pharmacological inhibition of RPS6KB1 and SIRT1 genetic silencing potentiate response to radiation-induced tumor cell growth inhibition. However, the precise mechanism how SIRT1 or RPS6KB1 sensitizes prostate cancer cells to radiation is unknown. In the current work, we have identified significantly decreased levels of GRPR (gastrin releasing peptide receptor) with RPS6KB1 silencing or pharmacologically inhibiting RPS6KB1 with berberine (BER). Furthermore, radiation treatment increased levels of pp70S6K and decreased nuclear SIRT1 levels. Interestingly, published reports show that SIRT1-mediated deacetylation of RPS6KB1 triggers its phosphorylation. GRPR is a 7-transmembrane protein that activates the phospholipase C and involved in inflammatory signaling. Based on our data and these published observations, we propose that GRPR (gastrin releasing peptide receptor) could facilitate SIRT1 and/or RPS6KB1-mediated radio-sensitization in prostate cancer cells.

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Abstract 2585

Living Light: A Complete GPCR Calcium Signaling Pathway Controls Bioluminescence in a Red Tide Algae

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Libin Ye

Dinoflagellates are a class of ecologically significant algae, with some species involved in harmful red tide blooms and other members acting as coral endosymbionts. Many of these dinoflagellates are bioluminescent, using a signaling pathway hypothetically linked to G-Protein Coupled Receptors (GPCRs); the GPCR is hypothesized to trigger flashes of light in response to shear stress. Genes identified from the whole transcriptome of Lingulodinium polyedra were annotated using the gene grouping tool eggNOG and several genes were annotated as GPCR candidates. BLAST and Conserved Domain search workflows were used to identify other pathway-relevant genes in the transcriptome, including all three components of a heterotrimeric G protein complex; the G-gamma candidate had not been identified previously. Phospholipase C and IPTR candidates were also identified, which along with previous literature presents a complete GPCR-mediated calcium signaling pathway common in action potential signals; this pathway was further supported by preliminary structure modeling and Protein-Protein Interaction prediction. Specific antisense oligonucleotides were designed to reduce gene expression via RNAseH mRNA digestion, then introduced to cells through lipofection. A single GPCR candidate—provisionally named Bioluminescence-Inducing Shear Receptor (BISR)—and a Phospholipase C candidate displayed bioluminescence reduction during the first bioluminescence cycle in a dose-dependent manner, along with increased brightness and peak shifting in the second cycle after knockdowns wore off. The results support the hypothesis that a GPCR mediates the bioluminescence of dinoflagellates. This research provides insight into the early stages of GPCR diversification and may lead to red tide management strategies which reduce bioluminescence to promote increased predation in a species-specific manner or prevent GPCR-mediated increases in red tide toxins.

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ASBMB

Abstract 2599

AMPK functions as a non-canonical GEF for Arf6 activation in a kinase-independent manner upon energy deprivation

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AMP-activated protein kinase (AMPK) is a crucial cellular nutrient and energy sensor that maintains energy homeostasis. AMPK also governs cancer cell invasion and migration by regulating gene expression and activating multiple cellular signaling pathways. ADP-ribosylation factor 6 (Arf6) can be activated via nucleotide exchange by guanine nucleotide exchange factors (GEFs), and its activation also regulates tumor invasion and migration. By studying GEF-mediated Arf6 activation, we elucidated that AMPK functions as a noncanonical GEF for Arf6 in a kinase-independent manner. Moreover, by examining the physiological role of the AMPK-Arf6 axis, we determined that AMPK activates Arf6 upon glucose starvation and 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) treatment. We further identified the binding motif in the C-terminal regulatory domain of AMPK that is responsible for promoting Arf6 activation and thus inducing cell migration and invasion of ovarian cancer cells. We previously also demonstrated that yeast Arf3 can be activated by Snf1, the yeast homologue of AMPK (Nature comms, 2015). These findings reveal an evolutionarily conserved role of AMPK in which its C-terminal regulatory domain serves as a non-canonical GEF for Arf6 activation during energy deprivation.

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Abstract 2603

Human steroid sulfatase deficiency induces keratinization of HaCaT cells by improving the expression level of E-cadherin

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Tae-uk Kwon, Yeo-Jung Kwon, Young-Jin Chun

X-linked ichthyosis (XLI) is a recessive genetic disorder of human skin caused by the genetic deletion of the steroid sulfatase (STS) gene. Deficiency of STS activity in XLI leads to intracellular lipid barrier malformation and delays keratinocyte degradation resulting in increasing corneodesmosomes. To understand how STS can regulate keratinization, the expression of cadherin proteins was determined because the cadherin proteins including E-cadherin may control the expression of keratinization-related proteins such as involucrin, loricrin, and TGM-1. In this study, we observed up-regulation of E-cadherin at mRNA and protein levels by knockdown of STS in human keratinocyte HaCaT cells. In addition, inhibition of STS enzymatic activity by STX64, a specific inhibitor, significantly enhanced E-cadherin expression. Furthermore, keratinization-related factors, including involucrin and loricrin, were increased in STS-knocked down cells. When cells were treated with TNF-α to induce STS expression, E-cadherin level was strongly repressed. Moreover, involucrin and loricrin were down-regulated when cells were treated with an E-cadherin antibody. Significant increases in epidermal and dermal levels were observed in STS-knockout mice compared to normal mice. Additionally, as the expression of E-cadherin increased, protein expression levels of markers for keratinization, involucrin, and loricrin were induced. In summary, these results suggest that STS deficiency may contribute to the desquamation which is induced by keratinization of human skin by inducing the expression of E-cadherin.

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Abstract 2629

Palmitoylation-dependent regulation of cardiomyocyte Rac1 signaling, myocardial hypertrophy, and oxidative stress

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We previously discovered that the small GTPase, Rac1, is palmitoylated at Cys-178 by the Golgi-localized S-acyl transferase, zDHHC3. Overexpression of zDHHC3 in cardiomyocytes induces Rac1 palmitoylation, GTP-loading, and hypertrophy, both *in vitro* and in cardiac-specific transgenic mice that develop lethal dilated cardiomyopathy and heart failure similar to transgenic expression of constitutively active Rac1. Importantly, mutation of the Rac1 palmitoylation site greatly reduces Rac1 GTP-loading compared to wildtype Rac1 in cardiomyocytes both *in vitro* and *in vivo*, suggesting palmitoylation-dependent regulation of Rac1 signaling activity. Translocation of Rac1 to the NADPH oxidase-2 (Nox2) complex at specific plasma membrane domains locally induces Nox2 oxidase activity, oxidative damage, and redox signaling in response to angiotensin-II (AngII) that is instrumental to the pathogenesis of cardiac hypertrophy and failure. However, molecular regulation of Rac1-dependent myocardial oxidative stress remains ill-defined. We found Rac1 palmitoylation, ROS levels, and hypertrophy were all enhanced in cultured cardiomyocytes treated with AngII, hearts of mice infused with AngII, and hearts of mice with cardiomyocyte-specific overexpression of the angiotensin-II type I receptor (AT1R) or the Rac1 S-acyltransferase, zDHHC3. Moreover, overexpression of an active Rac1 mutant in cardiomyocytes induced hypertrophy and oxidative stress that were mitigated by mutation of the Rac1 Cys-178 palmitoylation site. These data suggest palmitoylation of Rac1 is an essential regulatory mechanism governing stress-inducible cardiomyocyte hypertrophy and ROS production that could potentially be targeted to attenuate myocardial oxidative stress in heart failure. Ongoing studies are utilizing a novel cardiomyocyte-specific knock-in mouse model harboring a mutation in the Rac1 Cys-178 palmitoylation site to evaluate the necessity of Rac1 palmitoylation for AngII-induced cardiac hypertrophy and oxidative stress *in vivo*.

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Abstract 2632

Calcium and Insulin Signaling on the Outer Mitochondrial Membrane

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Daphnee Marcinek, Tim Locke, Shao-En Ong, Yasemin Sancak

Mitochondria have well-appreciated roles in functions outside of ATP generation, including signaling. Inspired by the role of lysosomes in functioning as signaling platforms for amino acid sensing, we wanted to know whether mitochondria can play a similar role for insulin and calcium signaling. To find such potential complexes, we optimized a proximity biotinylation technique known as BAR (Biotinylation by Antibody Recognition) to apply to the mitochondrial outer membrane in the absence and presence of these stimuli. Mass spectrometry analysis of these samples identified 1570 biotinylated proteins. 31 of these proteins significantly change their association with the mitochondrial outer membrane in response to these stimuli: 21 in response to calcium and 13 in response to insulin, with 3 responding to both stimuli. These proteins regulate a range of processes, including inter-organelle signaling, protein degradation pathways, and insulin resistance pathways. One of these hits, CSTB, has been shown to localize to the mitochondria in response to stimuli, showing the strength of our methodology in identification of mitochondria-associated proteins during signaling. Validation of our hits will elucidate novel protein interactions and be crucial for understanding metabolic signaling and homeostasis involving calcium and insulin.

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Abstract 2633

Determining the Role of EpiBrassinolide, a Plant Steroid, in Breast Cancer Cell Growth

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Breast cancer is the most prevalent form of cancer among non-smoking women globally, claiming the lives of over 600,000 women every year making the need for effective, noninvasive cancer treatment vitally important. Breast cancer tumors form as the result of uncontrolled cell growth and proliferation, which is partially controlled by the protein Glycogen Serine Kinase 3 (GSK3). GSK3 plays critical roles in apoptosis, tumor growth, cell invasion, and metastasis, making it a popular target for cancer therapies. When GSK3 is activated, cell death is promoted via the intrinsic apoptotic pathway and suppressed via the extrinsic pathway. Previous studies have noted EpiBrassinolide (eBR), a plant Brassinosteroid hormone, initiates apoptosis within MCF-7 breast cancer cells, making eBR a viable option for experimentation and future treatment. Brassinosteroids promote cell growth by inhibiting Brassinosteroid Insensitive 2 (BIN2), a Glycogen Serine Kinase. Brassinosteroids also lead to a decrease in the expression of anti-apoptotic proteins in breast cancer cells, and treatment with eBR has been shown to decrease the amount of viable breast cancer cells via apoptosis. This work identifies the GSK3 pathway as a potential target for eBR mediated apoptosis in MCF-7 cells. Alamar Blue assays indicated that eBR induced apoptosis most consistently at a 15 μM concentration. Western blotting revealed that GSK3 is phosphorylated and therefore activated after eBR treatment. This data connects eBR induced apoptosis with GSK3 signaling in MCF7 cells.

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Abstract 2653

Arachidin-1 Enhances the Anticancer Effects of Paclitaxel in Triple-Negative Breast Cancer Cells

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Triple-negative breast cancer (TNBC) is an aggressive type of cancer that is challenging to treat due to the lack of hormonal receptors used to target cancer cells; consequently, TNBC patients have high mortality rates. Investigating alternative therapies to increase survival rates for this disease is essential. Plant-derived compounds are being sought as potential adjuvants for common chemotherapy drugs, such as paclitaxel (Pac). To this end, the cytotoxic effects of the prenylated stilbenoids arachidin-1 (A-1) and arachidin-3 (A-3), and non-prenylated resveratrol (RES) were evaluated in human TNBC cells as potential adjuvants for Pac. A-1, alone or in combination with Pac, showed the highest cytotoxicity in TNBC cells. Apoptosis was further evaluated by measuring key apoptosis marker proteins, and cell cycle arrest. The results showed that A-1 decreased the Pac IC50 approximately 2-fold in TNBC cells. The synergistic combination of A-1 and Pac arrested cells in the G2/M phase and activated p53 expression. Our results demonstrated that A-1 in combination with Pac inhibited cell proliferation, and induced apoptosis through mitochondrial oxidative stress. These findings underscore the impactful effects of the prenylated stilbenoid A-1 as a novel adjuvant for Pac chemotherapy in TNBC treatment.

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Abstract 2654

Regenerating Islet Derived 3 (Reg3) Gene Expression Mediated by STAT3 is an Indicator of Colonic Dysplasia in a Mouse Model of NF-κB-dependent Colitis

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Siyuan Liang, Abrory Pramana, Guanying Xu, Yimeng Zeng, Yuan-Xiang Pan, Hong Chen

Objectives: Reg3 family of secretive proteins acts as a multifunctional secretory molecule with anti-inflammatory, antimicrobial, and probably immuno-regulatory effects. Recent studies indicate that colitis may be related to the expression level of Reg3. In this study, knockout mice with intestinal epithelial cells (IEC)-specific ablation of the hnrnp I gene (KO) were used as a mouse model of colitis to investigate the mechanisms of Reg3 involvement in the development of inflammation and colitis. IEC-specific KO of hnrnp I gene results in the activation of NF-κB signaling and the development of spontaneous colitis. The objective of the study is to examine the STAT3 signaling and the regulation of Reg3 gene expression in the colon of the KO mice during the induction of inflammation and colitis.

Hypothesis: We hypothesize that the expression of Reg3 is regulated through the interactions between STAT3 signaling and NF-κB signaling in a mouse model of colon inflammation and colitis.

Methods: Six weeks old WT and KO mice were sampled for body weight, colon weight, and length. The whole colon was opened and jelly-rolled from the distal to the proximal end for embedding. Total RNA and protein were extracted for quantification of gene expression by RT-qPCR; protein levels were quantified by ELISA or Western Blot. Colon tissue sections were examined for colon morphology and dysplasia using hematoxylin-eosin staining (H&E). The localization of proteins was analyzed using immunofluorescent staining. GATA3 was used as a marker to identify colon dysplasia. The human colorectal adenocarcinoma cell line HT-29 was used to analyze the interactions between STAT3 signaling and its downstream gene expression. TNF-α and IFN-γ were treated to trigger the phosphorylation of STAT3. Results were analyzed using the Mann-Whitney test. P less than 0.05 was considered significant.

Data and Results: Gene expression levels of Reg3b and Reg3g in KO mice were significantly lower than in WT mice. Further, the expression level of several cytokines in the Cxcl and Ccl families was significantly correlated with Reg3 gene expression in both WT and KO mice. Particularly, in KO mice, which generally had limited Reg3 expression levels, there is an increased incidence of invasive dysplasia in parts of the colon with an increased GATA3. Importantly, Reg3 expression levels in the dysplasia were significantly upregulated compared to the rest of the colon. Moreover, the KO mice with elevated Reg gene expression also exhibited increased phosphorylated STAT3, a key indicator of the activation of the STAT3 signaling. Furthermore, the expression level of the Reg gene was also significantly increased in HT29 cells with an activated STAT3 phosphorylation.

Conclusions: Our results indicate that under the activated NF-κB signaling condition in the colon epithelial cells in the hnrnp I KO mice, the mRNA expression levels of Reg3b and Reg3g were significantly downregulated. This suggests that NF-κB pathway had a regulatory effect on Reg. Further, there was a significant upregulation of REG3 and STAT3 protein expression in specific dysplasia regions that had high expression levels of GATA3 in KO mice, suggesting that REG may serve as an early indicator of colon dysplasia which may be mediated by STAT3 and GATA3 signaling. Therefore, dysregulation of the Reg family of proteins, along with specific cytokines in the Cxcl and Ccl families, is a critical biomarker for the early development of inflammation and colitis. Future research will focus on the mechanisms of actions of the NF-κB-STAT3-Reg3 signaling in the colon.

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ASBMB

Abstract 2678

Role of protein kinase B isoforms in the unfolded protein response

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The endoplasmic reticulum (ER) is a cellular organelle that is a site of several important functions including protein folding and secretion. Under conditions that generate ER stress, such as imbalance in the protein folding load and capacity, cells begin a signaling program called the unfolded protein response (UPR). UPR serves to restore homeostasis by triggering transcriptional pathways through stress sensing proteins in the ER membrane. In recent years, several groups, including ours, have shown that cell survival during ER stress is regulated by protein kinase B also known as Akt. Akt is a serine/threonine kinase that plays key roles in many important cellular processes including survival, metabolism, and migration. Although there are three isoforms of Akt (Akt1, 2, 3), most of the studies on Akt do not distinguish between the isoforms. While Akt1 and 2 are ubiquitously expressed, expression of Akt3 is restricted to specific tissues. This work aimed to identify the specific Akt isoform that regulates UPR. We used a colorectal adenocarcinoma cell line (DLD1) that only expresses Akt1 and Akt2. Lysates of cells treated with tunicamycin, which induces ER stress by inhibiting glycosylation of newly synthesized proteins, were analyzed by western blotting. Using isoform specific antibodies, we determined that while both isoforms showed activation upon induction of ER stress, Akt2 appeared to have a more appreciable increase in activity. Upon fractionating the cell lysates using differential centrifugation, we observed an ER-stress dependent increase in localization of Akt2 to the microsomal fraction. Together, these data imply that Akt2 is the main form of Akt involved in regulation of ER stress signaling. Our future experiments entail investigating the effect of Akt2 activation on UPR and any potential interactions between Akt2 and UPR effector proteins.

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Abstract 2679

Synthesis and analysis of 2,6-diarylidene cycloalkanones as Akt activators

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Serine/threonine protein kinase Akt has over 100 known substrates and is involved in regulation of several important cellular functions including proliferation, survival, metabolism, and migration. Due to its role in key cellular processes, it is a promising therapeutic target for diseases such as cancer and type 2 diabetes. As such, there is great interest in development of small molecule regulators of Akt activity. The focus of this project is to investigate 2,6-diarylidene cycloalkanones as a novel scaffold for modulation of Akt activity. Originally, 2,6-di-(4-pyridyl)methylidene cyclohexanone was reported to cause cell death by allosterically regulating the kinase activity and promoting degradation of Akt. However, our cellular data collected using western blotting based signaling analysis contradicts this model and suggests that Akt is in fact activated upon treatment with this compound. Interestingly, when we replaced cyclohexanone moiety with cyclopentanone, Akt activation was not observed. However, when the diaryl groups were varied, Akt activation was maintained. Our working theory is that the conjugation between the carbonyl and the aromatic rings is vital for Akt activation. To further study the mode of action of this scaffold, we will synthesize a library of 2,6-diarylidene cycloalkanones containing both symmetrical and asymmetric analogs and various substituents on the aromatic rings via Aldol condensation reactions. We will also perform direct binding studies with Akt and *in vitro* activity assays. This study will help elucidate the structural elements required for Akt activation by 2,6-diarylidene cycloalkanones and further evaluate the potential of these small molecules as Akt activators.

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Abstract 2682

Not quite FedEx: How are venom proteins packaged for delivery by the parasitoid wasp *Ganaspis hookeri*?

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Chris Lark, Nathan Mortimer

Parasitoid wasps are common pathogens of *Drosophila melanogaster*, and use venom proteins in order to overcome fly immunity. Venom proteins derived from the parasitoid wasp species *Ganaspis hookeri* alter the immune response mounted by immune cells known as plasmatocytes within infected *D. melanogaster* larvae. This venom activity is mediated by a unique venom-specific isoform of the SERCA (Sarco/endoplasmic reticulum Ca2+-ATPase) calcium pump. Venom SERCA activity inhibits the calcium burst normally mounted by plasmatocytes following infection, rendering them unable to melanize the foreign wasp egg. The mechanism by which SERCA and other venom proteins are transported into the host is not completely understood, but our preliminary evidence suggests that venom proteins are packaged into venom-specific vesicles known as venosomes. Ultracentrifugation of *G. hookeri* venom separates venom proteins into unique fractions. Nanoparticle tracking analysis and dynamic light scattering show the presence of vesicles in two of these fractions. Identification of protein content of these two fractions further supports the idea that venom proteins can be stored as cargo within venosomes. We hypothesize that venosomes allow venom proteins to gain access to plasmatocytes likely via the interaction between virulence factors present on venosome surface and host factors on the surface of plasmatocytes. In ongoing experiments, we are using vesicle imaging, mass spectrometry, SERCA activity assays, and spectrophotometric analyses to further characterize the putative venom vesicles.

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Abstract 2684

Lumit immunoassay: a versatile bioluminescent approach for probing cellular signaling pathway regulation through protein phosphorylation and degradation

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Monitoring cellular signaling events can help better understand cell behavior in health and disease states. Current immunoassays to study proteins involved in signaling can be tedious, require multiple steps, and are not easily adaptable to high throughput screening (HTS) format. Here we describe Lumit cellular immunoassay, a novel cell-based assay approach where immunodetection is combined with bioluminescent enzyme subunit complementation technology. It is solution based, and unlike other immunodetection techniques (ELISA or Western blot), does not include washing, liquid transfer, nor immobilization steps. Therefore, cells are lysed in the same well where antibody binding and luminescence generation steps occur. Lumit immunoassays take less than two hours to complete in a homogeneous "Add and Read" format and were successfully used to monitor the activation of multiple signaling pathways through specific nodes of phosphorylation in unmodified cells. A key advantage of this approach is that it does not require cell engineering. Therefore, the phosphorylation or the total amount of an intracellular protein may be detected at native levels in any cell type where it is expressed. We also tested deactivation of these pathways with different small and large molecule inhibitors including PROTAC degraders and obtained the predicted pharmacology. In one case study, we applied this technology to analyze RAS signaling pathway activation and inhibition through the detection of phosphorylated ERK. The Lumit pERK immunoassay was used to rank order potencies of allele specific inhibitors within cell lines harboring various activating KRAS mutations such as G12C, G12D and G12V. An inhibition profile was obtained indicating various potencies and selectivity of the inhibitors. Our results demonstrate that this technology can be broadly adapted to streamline the analysis of signaling pathways of interest or the identification of pathway specific chemical or biologic inhibitors.

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ASBMB

Abstract 2696

Distinct roles for clathrin and dynamin in real-time TrkA trafficking and NGF-induced pain

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Raquel Tonello, Nigel Bunnett

Nerve growth factor (NGF) is a key mediator of pain in both humans and rodents. NGF primarily signals via tropomyosin receptor kinase A (TrkA), a receptor tyrosine kinase (RTK) that triggers various signaling cascades (e.g., ERK) as well as undergoing endocytosis. Seminal findings demonstrated that NGF/TrkA signals from subcellular endosomes in sympathetic neuronal development, however few studies have directly investigated the role of NGF/TrkA endocytosis in nociception. Here, we aimed to investigate the kinetics, potency and mechanism of TrkA trafficking, probing the implications of endocytic inhibition on NGF-induced pain and TrkA signaling. Developing a sensitive method to monitor real-time RTK trafficking, TrkA was tagged at the C-terminus with a Renilla luciferase for bioluminescence resonance energy transfer (BRET). This acts as a BRET donor for a fluorescent marker of the plasma membrane (RGFP-CAAX). NGF induced TrkA removal from the plasma membrane within 5 min. This was concentration-dependent in both HEK293T cells and neuron-like CAD.a cells with a nanomolar potency. NGF-induced TrkA endocytosis was inhibited by pre-incubation (30 min, 37°C) with hypertonic sucrose (0.45 M) or a small molecule inhibitor of clathrin (Pitstop2, 50 μM). In contrast, agonist-induced TrkA trafficking was not blocked by dynamin inhibition using either pharmacological (Dyngo4a, 50 μM) or genetic (dominant-negative dynamin K44A) approaches. Instead, there was an enhanced constitutive level of TrkA localized at the cell surface upon dynamin inhibition. To investigate NGF-induced signaling *in vivo*, wild-type mice were monitored every 30–60 min for mechanical allodynia. Dyngo4a, Pitstop2 (50 μM/10 μl, intraplantar) or PBS were administered 30 min prior to injection of murine NGF (1 ng/10 μl, intraplantar). Whereas pre-treatment with Pitstop2 reduced NGF-induced sensitization for up to 60 min following NGF administration, there was no effect of dynamin inhibition on nociception. To directly measure TrkA-mediated kinase signaling, ERK signaling was monitored using a real-time FRET biosensor (EKAR) localized to the cytosol or nucleus. NGF induced a maximal ERK response within 5–10 min. Pre-incubation with sucrose or Pitstop2 reduced ERK signaling from both the cytosol and nucleus, while DnmK44A had no effect. These studies demonstrate a distinction between the endocytic mechanisms that govern TrkA localization and signaling. Whereas dynamin is involved in constitutive TrkA trafficking, clathrin mediates rapid NGF-induced TrkA endocytosis and modulates subcellular nociceptive signaling.

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Abstract 2701

Motility and More: the trypanosome flagellum as a platform for parasite signaling and motility.

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Trypanosoma brucei and other African trypanosomes are vector-borne parasites that cause substantial human suffering across sub-Saharan Africa. The *T. brucei* life cycle is punctuated by transitions between several developmental stages, each characterized by changes in morphology, metabolism, and gene expression profile, and occurring as the parasite moves through distinct host tissue compartments. Signaling pathways that drive movement between tissues and concomitant developmental stage transitions remain incompletely understood. Recent studies have started to fill this gap in knowledge, including elucidation of the parasite flagellum as a pivotal signaling center that houses not only machinery for motility, but also signaling systems that control parasite movement. A central role for the flagellum in both motility and signaling is advantageous, as parasite differentiation and motility are intimately integrated phenomena. Likewise, several studies have expanded our understanding of trypanosome motility mechanisms, including the parasite's ability alter flagellum-dependent movement in response to external cues and detailed 3D structure of the flagellum motility machinery. The discovery of trypanosome social motility, a cooperative motility behavior depending on cell-cell interactions, has been instrumental in many of these advances. In this presentation, we highlight some of the recent work that has transformed our understanding of signaling and motility functions of the trypanosome flagellum that are critical for transmission and pathogenesis of these deadly pathogens.

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Abstract 2723

Fig4 contributes to cellular homeostasis in *Saccharomyces cerevisiae* through a physical interaction with the p-21 activated kinase Ste20

Bethany Strunk, *Trinity University*

Anna King, Bonnie Lloyd, Khan Imran, Hannah Reeves, Harrison Hall, Corey Chung, Lauren Dotson

Fig4 is a phosphatidylinositol 3 5-bisphosphate (PI3,5P2) 5-phosphatase conserved in eukaryotes from yeast to humans. Mutations in Fig4 have been implicated in multiple human neurodegenerative diseases attributed to the dysregulation of PI3,5P2. Fig4 modulates levels of PI3,5P2 through direct association with the Fab1-Vac14-Fig4 complex contributing to the regulation of cellular processes including endo-lysosomal trafficking, ion channel gating, and TORC1 function. We have recently found that Fig4 disease-related mutations that impair its association with Fab1 and Vac14 promote a dominant growth advantage on rapamycin at elevated temperatures in *Saccharomyces cerevisiae*. This growth advantage requires the presence of Fig4, but is conferred independent of Fig4 catalytic function and does not require Vac14. Through investigation of the molecular mechanisms underlying this growth advantage, we have identified a novel interaction between the p21-activated protein kinase Ste20 and Fig4. Ste20 is required for the rapamycin-dependent growth advantage conferred by disease-related Fig4 variants. Moreover, Fig4 and Ste20 interact physically by immunoprecipitation and this interaction is enhanced with Fig4 variants that associate poorly with Fab1 and Vac14. We propose a model whereby Fig4 that is not tethered to the Fab1-Vac14-Fig4 complex is available to interact with Ste20 in a context-inappropriate manner. We hypothesize that the interaction observed between Fig4 and Ste20 reflects a normal association that is usually controlled by the regulated release of Fig4 from the Fab1-Vac14-Fig4 complex. A genetic interaction between Fig4 and the Drosophila Ste20 homolog Hippo was reported previously, raising the possibility that a functional interaction between Ste20 and Fig4 is conserved in metazoans. Our data suggest that Fig4 contributes cellular homeostasis in eukaryotic cells independent of its role in the production and turnover of PI3,5P2.

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