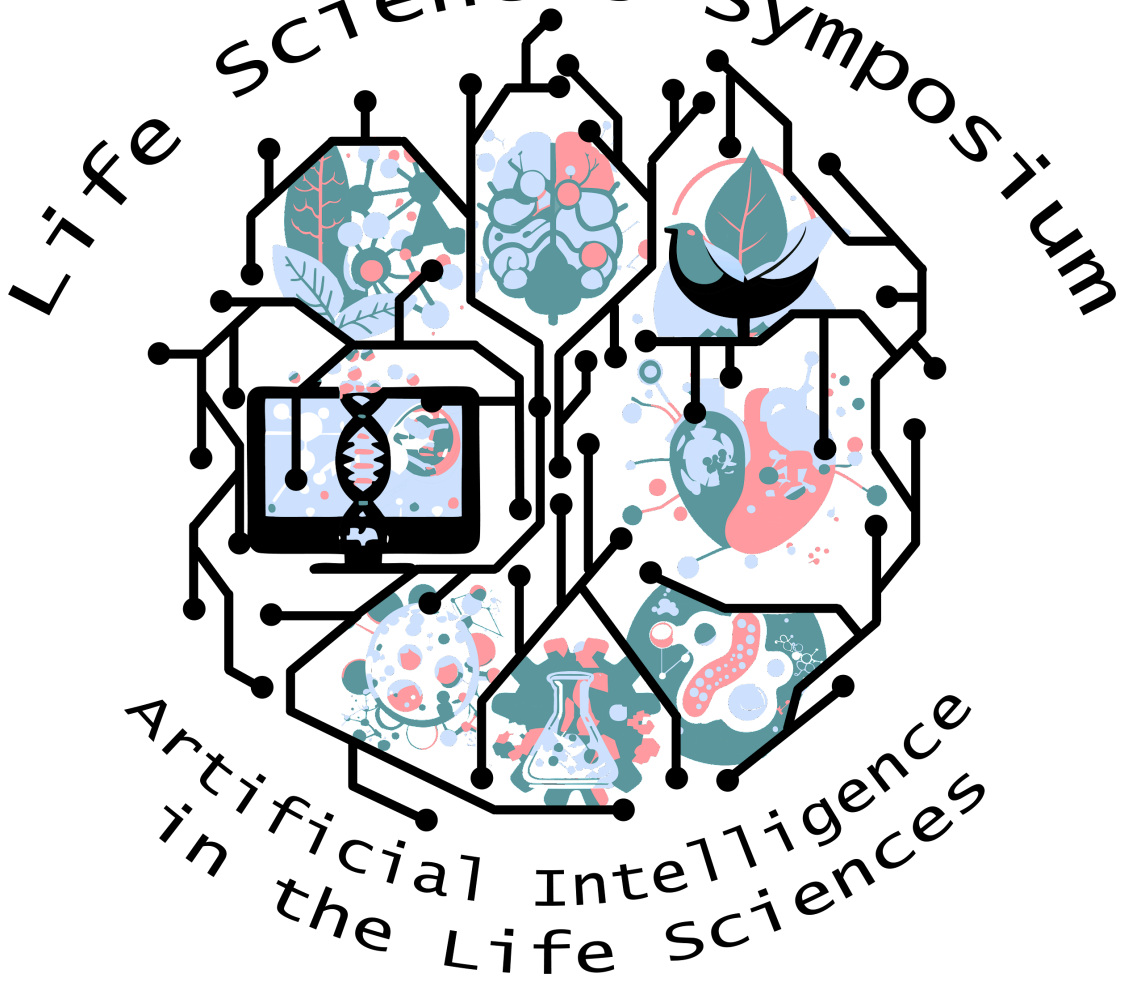
8th Annual

2024 Life Sciences Symposium



May 17th

8am - 6pm

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Artificial Intelligence in the Life Sciences

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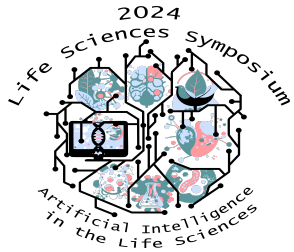
Abstract Index

Graduate Student Oral Presentations

Postdoctoral Fellow Oral Presentations

Poster Presentations

Animal Science, Agriculture and Plant Biology



Chemistry, Biochemistry, and Structural Biology

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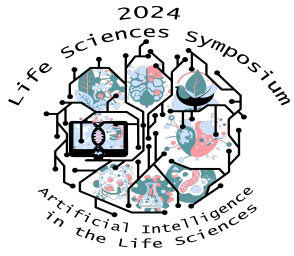
Microbiology and Virology

Neuroscience

Physiology and Nutrition



Xiaoling Chen

Abstracts

Graduate Student Oral Presentation

1 **The pod doesn't fall far from the tree: Parental effect on seed lipid content, composition, and thermal characteristics in *Theobroma cacao* L.**

Evelyn Kulesza, Helene Hopfer, Gregory Ziegler, Juan Calle-Bellido, Chelsea Roberts, Pathmanathan Umaharan, Siela Maximova, Mark Guiltinan
Department of Plant Biology, Penn State University

The seeds of *Theobroma cacao* L., known as the chocolate tree, hold immense value due to their rich content and composition of cocoa butter. Cocoa butter, the lipid component found in these seeds, is highly prized for its versatile applications in chocolate making, cosmetics, and pharmaceuticals, due to its unique thermal properties. The lipid content and composition of cocoa butter are influenced by various factors such as genotype, origin, and environmental conditions in which cacao is cultivated. However, the full extent of natural variation in cocoa butter remains inadequately explored, requiring further research to comprehensively characterize its diversity. Parental genotypes play a significant role in shaping seed traits. This is seen primarily through the inheritance of chromosomal genes from both parents, as well as maternal transmission of mitochondria and chloroplasts, and with resource allocation from the maternal plant during fruit development. Although the influence of parental genotypes on seed lipid traits is well-documented across many plant species, this aspect has not been extensively studied in cacao. In this study, we examined seeds resulting from reciprocal crosses between cacao genotypes PA150 and SCA6, alongside seeds from open-pollinated fruits of PA150, to investigate the maternal and paternal influences on cocoa butter. We employed various analytical techniques to quantify multiple physical traits of cocoa butter, including fat content measured via nuclear magnetic resonance (NMR), triglyceride composition assessed using gas chromatography with flame ionization detection (GC-FID), and thermal properties such as melting and recrystallization temperatures and enthalpy measured via differential scanning calorimetry (DSC). The composition and proportion of triglycerides significantly affect the thermal properties of cocoa butter products, impacting factors like melting and crystallization. By analyzing the triglycerides present in cocoa butter, we can correlate these properties with how different triglycerides assemble within the crystalline structure. Our findings revealed considerable variation in fat content among seeds from the three different fruit types. Additionally, the composition of several triglycerides and physical traits varied significantly among the progeny of these crosses. Importantly, the directionality of the cross was found to have a significant impact on cocoa butter traits examined in this study. These results highlight the potential for selecting specific maternal and paternal genotypes to produce cocoa butter with

tailored functional traits, beneficial for culinary, cosmetic, and pharmaceutical applications. Moreover, these findings provide insights that could inform planting and pollination strategies aimed at developing new cacao varieties with cocoa butter customized to meet desired functional specifications.

2 **DICER-LIKE 1 is Crucial for Generating Trans-Species MicroRNAs in the Parasitic Plant *Cuscuta campestris***

Ya-Chi Nien, Michael J. Axtell
Department of Biology, Penn State University

The parasitic plant Dodders (*Cuscuta spp*.) are vampires of the plant world. Their “fangs”, or haustoria, bite through the host plant’s skin and suck out water and nutrients. At the haustorial interface, *C. campestris* sends short strands of RNA, called microRNAs, into the host, manipulating host genes. However, it is largely unknown how these interface-induced microRNAs are made. Most microRNA precursors are processed by a protein called Dicer-like 1 (DCL1), into mature 21- or 22-nucleotide functional form. We speculate that DCL1 is also involved in processing interface-induced microRNAs in *C. campestris*. If this is true, decreasing *C. campestris* DCL1 (CcDCL1) should subsequently hinder the accumulation of these microRNAs. To test this, we employed Host-Induced Gene Silencing, infiltrating artificial microRNA targeting CcDCL1 into the host, *Nicotiana benthamiana*, and allowing *C. campestris* to feed on the host. Successful delivery of the artificial microRNAs achieved a remarkable 50% knockdown of CcDCL1 within a week. Excitingly, the tested trans-species microRNAs were significantly repressed upon reduced CcDCL1, while infiltration of artificial microRNAs against host DCL1 had no effect. The result underscores the essential role of *C. campestris* DCL1 in trans-species microRNA processing, occurring within the parasite and independent of host machinery. Moreover, the diminished CcDCL1 impeded *C. campestris* attachment by 40-50%, indicating that disrupting DCL1 poses a challenge for *C. campestris* in engaging parasitism. Our study gains significance as *Cuscuta* is among the USDA's Top Ten most noxious weeds, causing potential 80% yield losses if uncontrolled. By being the first to deliver RNA interference into *Cuscuta* through agroinfiltration in *N. benthamiana*, we have pioneered a method that facilitates rapid testing of numerous parasite genes, boosting the efficiency of *Cuscuta* research. If trans-species microRNAs production can be halted, it may offer new strategies to combat *Cuscuta* infestation, creating a vampire-free zone for our crops.

3 **Monitoring of solitary bee foraging and nesting behaviors to inform population models using an automated IoT and AI based monitoring system**

Edward Amoah, Santosh Sanjel, Christina Grozinger
Department of Entomology, Penn State University

Osmia cornifrons, also known as mason bees, are vital pollinators for spring-blooming fruits like apples and blueberries. Some studies have shown that *Osmia cornifrons* can be up to 80 times more effective than *Apis Mellifera* at pollinating crops like apples. This study will investigate the influence of temperature, precipitation, and landscape on *Osmia cornifrons* reproductive

success. This study will utilize an automated AI-powered monitoring system to monitor the foraging and nesting activities of *Osmia cornifrons* from a solitary bee hotel. The automated monitoring system consists of an IoT-based camera module that will record the foraging and nesting activities of the bees in the field and a computer vision system that will analyze the videos to extract the foraging and nesting activities for analysis. The monitoring system will monitor *Osmia cornifrons* foraging and nesting behavior at multiple sites with different landscape and foraging qualities. After nesting the bee hotels from the various sites will be taken to the lab for further analysis. The cells per nest and the sex ratio in each nest will be evaluated. The nesting data and the monitoring data will enable us to investigate the influence of temperature, precipitation, and landscape on the rate of eggs laid by females and the sex ratio of the eggs. This AI-powered monitoring system will further our understanding of the potential impact of climate change and landscape on *Osmia cornifrons*, an important alternate pollinator for agriculture in PA.

4 **The smell of danger: Transcriptomic reprogramming in tall goldenrod (*Solidago altissima* L.) following exposure to the pheromone of a gall-inducing herbivore**

Robert J. Witkowski, Lily A. Sudol, Eric C. Yip, John F. Tooker, Tanya Renner
Huck Institutes of the Life Sciences, Penn State University

Plants are highly sensitive to chemical cues in the environment–-they recognize both friend and foe largely by chemical signals. In their long co-evolutionary history with insects, many plant species have entered into an “arms race” with herbivores, employing sophisticated chemical defenses to discourage or defeat insect feeding. When plants perceive chemicals associated with a specific herbivore, they ready their chemical defenses against a possible later attack from that herbivore, a phenomenon called priming. Priming mediates the interactions of tall goldenrod (*Solidago altissima* L.) and the goldenrod gall fly (*Eurosta solidaginis* Fitch), a specialist obligate parasite that feeds on goldenrod stems. Male gall flies emit a volatile pheromone to attract mates, but this airborne chemical can be perceived by “eavesdropping” goldenrod plants. Moreover, *S. altissima* plants show heightened induction of defense hormones, e.g., jasmonic acid, during damage after being exposed to *E. solidaginis* pheromone. Priming likely entails wide gene expression rearrangement–the stockpiling of chemical “weaponry”–in preparation for a future attack; however, the specific genes induced by this process have been postulated but not confirmed. What molecular pathways are activated after *S. altissima* perceives the emission of male *E. solidaginis*? How does priming augment chemical defense during later insect damage? To answer these questions, we conducted a combined transcriptomic and chemical study, the first of its kind in this classic model ecology system. We exposed *S. altissima* plants to *E. solidaginis* pheromone then subjected them to herbivory from a generalist caterpillar for 48 hours. We extracted phytohormones and RNA from damaged plant tissue at five time points during that period for gas chromatography-coupled mass spectrometry and Illumina RNAseq, respectively. *S. altissima* hormone response during damage appeared to be accelerated by earlier exposure to the *E. solidaginis* cue. Our de novo transcriptome and gene expression analysis showed differential regulation of defense-related genes between primed and unexposed plants. A suite

of gene ontology (GO) terms were enriched in primed plants, including jasmonic acid metabolism, response to other organism, and biotic stress signaling. We leveraged a model ecology system to shed valuable light on the dynamics of plant defense and how it changes after a threat signal.

5 **A synthetic microbiota designed through meta-analysis provides insight to community function in *Clostridioides difficile* resistance**

Shuchang Tian, Min Soo Kim, Jingcheng Zhao, Kerim Heber, Fuhua Hao, David Koslicki, Andrew D. Patterson, Jordan E. Bisanz
Department of Biochemistry and Molecular Biology, Penn State University

Clostridioides difficile (Cd) is a common cause of recurrent antibiotic-associated diarrhea and colitis for which long-term efficacious and safe treatments are needed. Antibiotics are initially effective in treating CDI; however, their persistent disruption to the microbiome frequently leads to recurrent infection. Fecal microbiota transplant (FMT) is an effective treatment but the reliance on human donors makes FMT composition intrinsically irreproducible and may carry undesirable adverse effects on host physiology and/or transmit undesirable microbes such as multidrug-resistant pathogens. We created a synthetic FMT (sFMT) using a meta-analysis from 12 studies (N=899). A machine learning classifier predicted Cd colonization using microbiome composition, revealing taxa anti-correlated and positively correlated with Cd. We built a lab-derived synthetic microbial community from the anti-correlated predictors—sFMT1 from 37 bacterial strains and found it was capable of maintaining stability when cultured *in vitro* and during the colonization of germ-free mice. Another lab-derived community that positively correlated with Cd (proCD) showed no effect on *C. difficile* growth in vitro while sFMT reduced the outgrowth of *C. difficile*, which further demonstrates the specificity of sFMT's composition to maintain its efficacy. To determine sFMT's mechanism of action, each member of the sFMT1 was screened for plausible mechanisms including bile acid metabolism, short-chain fatty acid production, and Stickland fermentation through which sFMT may inhibit *C. difficile* colonization. Variants of sFMT1 by functional strain reduction showed one bacterial member capable of Stickland fermentation is sufficient and necessary to offer protection against infection outcomes while bile acid metabolism is dispensable for the efficacy of sFMT1. Our data demonstrate that meta-analysis combined with the usage of artificial intelligence is a viable strategy to design ecologically-informed synthetic microbiota which are valuable tools for both basic science research and potential tools for translational applications. Furthermore, the tractability of this synthetic community allows the discovery of the necessary and sufficient pathway to suppress C. difficile infection.

6 **IS1 mediated PMB heteroresistance in *E. coli* B strains**

Aditi M. Ranade, Michael Maybin, Nicolas Gisch, Uwe Mamat, Timothy C. Meredith
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Antimicrobial resistance (AMR) is a growing cause of concern across the globe and has resulted in the need for an improved understanding of the mechanisms leading to the emergence of AMR

infections and developing new drugs to combat drug resistant bacteria. Currently, polymyxins (colistin and polymyxin B) are the last line of defense against Gram-negative infections. Polymyxins are positively charged lipopeptides that interact with the negatively charged lipopolysaccharides (LPS) on the outer surface of the outer membrane of Gram-negative bacteria, disrupting the permeability barrier and resulting in bacterial cell death. However, Gram-negative bacteria have developed ways to evade polymyxins by modifying the lipid A component of the LPS by the addition of phosphoethanolamine (PEtN) via eptA or aminoarabinose (L-Ara4N) moieties via the arn operon. This has resulted in the emergence of polymyxin resistant clinical isolates of gram-negative pathogens. To add to this problem, antimicrobial resistance is often accompanied by the phenomenon of heteroresistance, defined as the presence of a subset of bacterial population that has an increased resistance to the drug compared to the rest of the population. Heteroresistance results in mischaracterization of resistant/susceptible populations and can often lead to treatment failures. In this study, we aimed to address the cause of polymyxin B heteroresistance seen in gram-negative bacteria and as a result, we have identified a novel mechanism of polymyxin resistance dependent on the presence of the *arn* operon, involving large, unstable, tandem genome segment amplifications flanked by insertion sequences. We employed MIC testing via the microbroth dilution method and measured the frequency of resistance via Luria Delbruck Fluctuation assay in isogenic strains of *E.coli* BL21(DE3). While *eptA* or *arnA* deletion resulted in a comparable drop in the MIC, the frequency of resistance of the *ΔarnA* mutant was around 10-fold lower than that of the *ΔeptA* mutant, suggesting that the arn operon might play an important role in the polymyxin heteroresistance phenotype. To test this, we derived polymyxin resistant mutants in a *ΔeptA* background to enrich for arn dependent routes of resistance. Electrospray ionization Mass Spectrometry (ESI-MS) was employed to determine the structural changes in the LPS purified from the resistant mutant from the susceptible parent. Whole genome sequencing and RNAseq were used to determine the genetic and transcriptomic differences between the parent and resistant mutant strains. ESI-MS revealed the increased abundance of L-Ara4N modified lipid A in mutant strains compared to the parent. Concurrently, whole genome sequencing revealed the presence of large genome segment amplifications which not only contained the *arn* operon but were also flanked by insertion sequences. RNAseq experiments confirmed the increase in the *arn* operon transcripts in the resistant mutants compared to the parent. We also identified upregulation of genes with putative roles in persister cell formation in the mutant strains. Overall, this study unearths a novel mechanism by which Gram-negative bacteria can leverage the LPS modifying genes to gain transient resistance to polymyxins. Follow up studies will include the regulation and mechanism of insertion sequence mediated genome segment duplications, and whether this mechanism prefers *arn* operon to *eptA* to gain polymyxin resistance.

7 **Leveraging FracMinHash Containment for Genomic dN/dS Estimations**

Judith Rodriguez, Mahmudur Rahman Hera, David Koslicki
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Increasing availability of metagenomic data demands algorithmic approaches that can efficiently

and accurately conduct downstream metagenomic analyses. Analyses such as evaluating selection pressures within and across genomes can reveal developmental and environmental pressures. Traditionally, selection pressure has been calculated using the ratio of nonsynonymous to synonymous mutations, known as dN/dS. Nonsynonymous mutations are mutations in a codon that mutate the resulting residue and the synonymous mutations are mutations in a codon that do not change the resulting residue. The proportions of these mutations to their sites are calculated and are subsequently used for the dN/dS ratio. Calculating dN/dS at a gene level can be straightforward yet can become a challenging computational endeavor when dealing with larger datasets such as metagenomic data, primarily due to demanding processing time in sequence alignments. Consequently, traditional methods of estimating dN/dS ratios become less scalable for larger sequences particularly when dealing with extensive sequences like entire genomes. This limitation constrains their utility primarily to smaller scales, such as individual genes. Here, we present FracMinHash (FMH) Omega, an alignment-free approach to estimating dN/dS at a genomic level for metagenomic data that utilizes the FMH hashing technique. FMH is a hashing technique where k-mers from a sequence of interest are generated and a simpler representation of these k-mers are sketched. Previous work in our lab has shown that FMH can accurately estimate average nucleotide identity (ANI) and average amino acid identity (AAI), which can be combined to estimate dN/dS. The regions of ANI can be leveraged to estimate regions contributing to differences occurring which can correspond to evolutionary events such as gene lost. Our results suggest that employing FMH to estimate dN/dS is scalable to the Genome Taxonomy Database (GTDB), allowing us to perform pairwise estimations of dN/dS for 85,205 genomes within 5 hours. Furthermore, our approach is comparable to traditional dN/dS methods using synthetic and real word data. In our experiments with synthetic data, representing sequences subject to positive and negative selection across various mutation rates, we observed estimations consistent with those derived from the NG86 method for calculating dN/dS. Similarly, when applied to real-world genomic data pertaining to S*ynechococcus* and *Prochlorococcus* bacterial strains, our approach yielded dN/dS interpretations that align closely with existing literature interpretations of phylogenomic tree analyses. In summary, we present a novel method for estimating dN/dS at a genome level that is scalable beyond gene-level estimations while demonstrating comparability with real-word biological data.

8 **Developing coral-inspired artificial host cells for living algal symbionts**

Dharani Abeysinghe, Christine Keating
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Coral reefs, often referred to as “rainforests of the sea”, are some of the most diverse ecosystems in the world. These reef building corals have a symbiotic relationship with dinoflagellate algae called *Symbiodinium*. The algae produce nutrients that are essential for coral growth and calcification. In turn, the coral provides the algae with a sheltered habitat and supports algal photosynthesis. Tragically, this coral-algae mutualism, which is the basis for the existence of all shallow-water coral reefs, is at great risk due to climate change. Heat stress and ocean acidification cause coral bleaching (loss of algal symbionts) via mechanisms that remain poorly

understood. Advancing fundamental understanding of coral-algae symbiosis is crucial for predicting and combating the effects of climate change on coral reefs and related marine ecosystems. This work focuses on developing coral-inspired artificial cells which serve as a test bed for investigating the basics of coral-algae symbiotic relationships while clarifying the essential role(s) of algal symbiont vs host. This is achieved through simplified and controlled chemistry, omitting the complexities of the real biological system to isolate and study how a specific change in the host environment impacts the algae. We mimic algae-hosting compartments in corals, namely symbiosome vacuoles, using liquid-liquid phase separated condensates loaded with enzymes governing biochemical functions. Confocal microscopic data demonstrates the feasibility of encapsulating living algal cells inside these artificial symbiosome vacuoles. Effective quantum yields of photosynthesis measured by pulse amplitude modulated fluorimetry indicate the compatibility of the artificial host cell environment with algal photosynthesis. By rationally varying the chemical composition and morphology of these artificial coral cells, we aim to learn the impact of key factors (i.e., temperature, acidity, local carbon dioxide availability) on survival and photosynthesis of compartmentalized dinoflagellate algae. This in turn will help identify mechanisms of coral bleaching and enable new approaches to build "climate-proof" coral reefs. Furthermore, this hybrid live algae-artificial coral system could be adapted to other energy related biotechnologies such as organic carbon capture, e.g., to advance algal-based biofuels by supporting desired algal functions via artificial host cells.

9 **Scalable high-throughput aspiration-assisted spheroid bioprinting for rapid tissue fabrication**

Myoung Hwan Kim, Yogendra Pratap Singh, Nazmiye Celik, Miji Yeo, Ibrahim Tarik Ozbolat
Department of Biomedical Engineering, Penn State University

Bioprinting is a promising technology for tissue engineering and regenerative medicine. However, current bioprinting techniques face a critical limitation in achieving physiologically relevant cell densities, impeding the success of developing functional tissues that mimic native structures. Recently, an innovative technique known as ‘aspiration-assisted bioprinting (AAB)’ has been developed, facilitating control over the shape, architecture, and distribution of bioprinted tissue. This technique enables precise picking and placement of different sizes of biologics into or onto a gel substrate with minimal cellular damage, achieving high positional precision. However, the primary limitation of AAB is its reliance on a single nozzle for bioprinting one spheroid at a time, which significantly prolongs the process and poses challenges to scalability. To address these limitations, this study introduces a novel technology termed high throughput aspiration-assisted bioprinting (HT-AAB), utilizing a digitally controlled nozzle array (DCNA). The DCNA allows for selective control of individual nozzles through solenoid valves and pressure/vacuum sensors in customized software, enabling rapid, high-throughput bioprinting with precise positioning and reduced manual intervention. Spheroids, used as building blocks due to their ability to replicate complex cellular microenvironments, can be selectively loaded onto nozzles and released at designated positions. By controlling the aspiration pressure, the DCNA platform significantly reduces bioprinting time, allowing for the precise placement of approximately 100 spheroids in less than 5 minutes. As a proof of concept, we propose a regenerative strategy for

craniomaxillofacial (CMF) defects through intraoperative bioprinting of HT-AAB using a hybrid process that integrates extrusion-based bioprinting with the DCNA platform. Patient-specific bone grafts are bioprinted using miRNA-transfected osteogenically-committed bone spheroids, demonstrating successful bioprinting onto rat calvarial defect area with two different densities of spheroids, leading to homogeneous bone regeneration at week 6. Furthermore, the feasibility of scalable tissue fabrication was demonstrated by fabricating a 1 cm^3 size cartilage tissue construct containing 576 spheroids, showcasing the capability of DCNA for larger-scale applications. In summary, the DCNA facilitates the fabrication of patient-specific and defect-specific constructs for CMF regeneration as well as the scalable tissue fabrication, representing a significant advancement in bioprinting and extending its implications to personalized treatments and clinical translation in regenerative medicine.

10 **Vitamin D deficiency impacts the development of immunity from embryonic to adulthood**

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Vitamin D is an essential nutrient and hormone that is classically recognized as a regulator of calcium and mineral homeostasis. Immune cells express the vitamin D receptor (VDR) and are therefore vitamin D targets. To trace expression of the VDR, we created a transgenic VDR reporter mouse that produces the tdTomato fluorescent protein upon expression of the VDR. To examine the effect of vitamin D deficiency on immune cells, we utilized a dietary vitamin D deficiency model and genetic vitamin D deficiency model, CYP27B1 and VDR knockout (KO) mice. VDR/tdTomato+ expression was found in the fetal liver of embryonic (E) day 15.5 offspring. Hematopoietic stem cells from vitamin D sufficient moms had significantly higher VDR/tdTomato+ expression in the fetal liver than the offspring from vitamin D deficient mothers. Both dietary and genetic vitamin D deficiency models showed decreased frequency of hematopoietic progenitor cells in the fetal liver at E15.5. At birth, immune system development shifts from the fetal liver to the bone marrow and post-natal immune development becomes impacted by environmental factors such as colonization by microbiota. Three patterns of VDR expression were identified in postnatal immune cells. CD4+ T cells and natural killer cells showed high expression of VDR/tdTomato+ at birth that remained high through adulthood. Innate lymphoid cells (ILC) 2 were predominately VDR/tdTomato- at birth and remained VDR- in adulthood. Lastly, ILC1, ILC3, and CD8+ T cells expressed low VDR/tdTomato+ at birth which increased with age. Interestingly, the adult offspring from vitamin D deficient mothers had reduced expression of VDR/tdTomato+ in the spleen compared to the adult offspring of vitamin D sufficient mice. Vitamin D treatments that began at weaning (3 wks of age) through adulthood (8 wks of age) of vitamin D deficient mice restored VDR/tdTomato+ expression to that of vitamin D sufficient mice. Whether the vitamin D interventions at weaning can overcome the functional effects of maternal vitamin D deficiency in the offspring will be reported on. Developmental vitamin D deficiency affects the ability of the offspring to express the VDR and be regulated by 1,25D in both fetal and postnatal phases of immune cell development.

11 **The short-term effect of increasing doses of palmitic and stearic acid on milk fatty acid profile**

and milk fat thermal properties in Holstein cows

A.N. Staffin, G.R. Ziegler, K.J. Harvatine
Department of Animal Science, Penn State University

Palmitic and stearic acid are commonly fed to dairy cows but there is limited data on their effects on thermal properties of milk fat, especially at different dose levels. Recently, consumers voiced concerns about their butter not being soft at room temperature and blamed palmitic acid supplementation. These concerns made international news under the name “Buttergate'' and highlighted a need for further research. Our hypothesis was that increasing palmitic acid intake would linearly increase palmitic acid in milk fat and increase percent solid of butter oil at 20°C, whereas increasing stearic acid intake would increase both stearic and oleic acid in milk fat and not change the percent solid of butter oil at 20°C. Twelve Holstein cows (106 ±31 DIM) were arranged in a replicated 3x3 Latin square design with a dose escalation design within period and a ≥10 d washout between periods. Treatments included a no-supplement control (CON), a fatty acid (FA) supplement high in palmitic acid (PA; > 80% palmitic) and a FA supplement high in stearic acid (SA; 80% stearic and 10% oleic). The FA supplements were fed at increasing doses every 4 d targeting 150, 300, 500, and 750 g/d. Milk samples were collected on d 3 and 4 of each dose, composited, and butter oil extracted from fat cake by centrifugation. FA profile was analyzed using GLC and melting properties using direct scanning calorimetry. Data were analyzed by ANOVA with preplanned contrasts testing CON vs PA and CON vs SA at each dose level. PA progressively increased palmitic acid in milk fat while SA progressively increased both stearic and oleic acid ($P < 0.05$). At 750 g, PA increased milk palmitic acid 5.7 percentage units compared to CON (36.8% vs 30.1%; $P < 0.001$) while SA increased milk stearic acid 2.4 units and oleic acid 3.0 units compared to CON (11.8% vs 9.4% and 20.2% vs 17.2%, respectively; $P < 0.001$). The percent solid of butter oil at 20°C was linearly increased by PA but was decreased by SA. At the 750 g dose PA increased percent solid 5.2 percentage units and SA decreased it 3.7 percentage units compared to CON ($P < 0.001$). In conclusion, increasing palmitic acid intake increases percent solid of butter oil at room temperature while increasing stearic acid modestly decreases it, with differences likely due to differences in mammary desaturase enzyme activity.

12 **Sex differences in brain activity and functional connectivity impact of GLP-1 and amylin in awake rats**

Tanzil Arefin, Stina Börchers, Doris I Olekanma, Morgan R Sotzen, Suyeun Byun, Nanyin Zhang, Karolina P Skibicka
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Gut-produced glucagon-like peptide-1 (GLP-1) and pancreas-made amylin produce potent reductions in ingestive behavior, and alter brain activity, directly and indirectly, to achieve this outcome. While for both peptides a direct action in the hindbrain and the hypothalamus is expected, few rodent studies examine their impact on the whole brain activity, and those that do, test anesthetized male rats. However, both sex and anesthesia may significantly alter the influence of feeding controlling molecules on brain activity. Therefore, here we set out to

investigate the effect of GLP-1 and amylin on brain activity and functional connectivity (FC) in awake adult male and female rats using resting-state functional magnetic resonance imaging (rsfMRI). Rats were habituated in a mock scanner over one week, and then tested with in-scanner intraperitoneal infusions of vehicle, GLP-1, and amylin. Subsequently, each rat was returned to their home-cage and food intake was measured for 1h to examine the relationship between the effects of the feeding peptides on ingestive behavior and brain activity, as well as FC. Very little overlap in brain activation patterns by GLP-1 or amylin was found between males and females. FC matrices also indicated a sex divergent effect of amylin and GLP-1. Most importantly correlation analysis between FC and feeding behavior revealed that different brain areas potentially drive reduced food intake in male and female rats. Our findings underscore the distributed and distinctly sex divergent neural network engaged by each of these anorexic peptides and suggest that different brain areas may be the primary drivers of the feeding outcome in male and female rats. Our data further highlight prominent activity and FC alterations in brain areas not typically associated with feeding behavior in both sexes. This impact should be further investigated as it may either indicate novel feeding centers or alternatively suggest behaviors outside of feeding and metabolism may be impacted by these substances. The latter question is of potential translational significance as analogues of both GLP-1 and amylin are clinically utilized.

Postdoctoral Fellow Oral Presentations

13 **Short-term caloric restriction alters the microbiome modulating resistance to *Clostridioides difficile* infection**

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Department of Biochemistry and Molecular Biology, Penn State University

Clostridioides difficile is an opportunistic pathogen leading to diarrhea, colitis, and potentially death. The primary treatment for *C. difficile* infection is antibiotics; however, the use of antibiotics disrupts the gut microbiome and therefore increases vulnerability to *C. difficile*, resulting in recurrent *C. difficile* infection. Diet is a major factor modifying the gut microbiota and our previous work has shown that a 2-month 800 kcal/day liquid diet disrupted the microbiome and modified susceptibility to *C. difficile*. The objective of the current study was to contrast the effects of dietary form and duration on gut microbiota and colonization resistance to *C. difficile*. We conducted a randomized, cross-over study of 10 healthy individuals consuming either a liquid very-low-calorie diet or a solid-food nutritionally matched diet over 5-day intervals with a 5-day wash-out period in between. Interestingly, both short-term liquid and solid diets had significant effects on microbiota composition with an increased impact of highly bioavailable liquid diets. Fecal microbiota from the human participants before and after dietary intervention was transplanted to germ-free mice which were challenged with *C. difficile*. Contrasting our previous results, both 5-day diets increased colonization resistance to *C. difficile* infection. We next designed a caloric restriction (CR) model in conventional mice and confirmed that short, but not

long-term CR, protected against *C. difficile* infection. To confirm these findings in a tractable model, humanized mice were randomly assigned to three CR levels (100, 60, and 30% ad libitum) for 1 week and then their microbiota was transplanted into recipient diet-naive germ-free mice. Short-term CR significantly increased *C. difficile* resistance in both dieted and diet-naive recipient animals which was robustly associated with an increase in a *Bacteroides caccae* strain. We isolated the representative strain of *B. caccae* and confirmed that the administration of *B. caccae* could protect against *C. difficile* infection in vivo. Furthermore, genomic analysis of *B. caccae* revealed its potential in degrading host-derived glycans, confirmed by *in vitro* assays demonstrating that *B. caccae* could utilize mucin to survive during short-term CR producing short-chain fatty acids (SCFAs). These findings reinforce the interplay between diet and microbiome composition in affecting host health while pointing towards new strategies for infectious disease management.

14 **Unlocking Biocatalytic Potential: Isothiocyanate Radical Transfer by BesD Nonheme Iron Halogenase**

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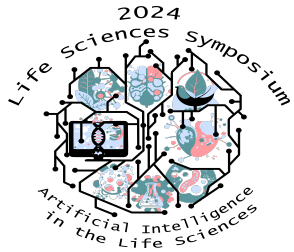
Isothiocyanates (ITCs) are commonly found in natural products and bioactive compounds, characterized by the R–NCS structure. Compounds containing isothiocyanate have been noted for their diverse pharmacological effects, including antioxidant, antimicrobial, antibacterial, anti-inflammatory, antifeedant, anticancer, antiproliferative properties, and enzyme inhibition against HIV. They are naturally occurring molecules derived from glucosinolate precursors found in cruciferous vegetables such as broccoli, watercress, Brussels sprouts, cabbage, Japanese radish, and cauliflower. Many isothiocyanates, whether natural or synthetic, exhibit anticarcinogenic properties by inhibiting carcinogen activation and enhancing detoxification processes. Additionally, isothiocyanates serve as essential reagents in organic chemistry, particularly in synthesizing various heterocycles like thiazoles, thiadiazoles, triazoles, benzimidazoles, dithiolane, spiro-fused oxazolines, triazines, and oxazines, among others. Although C–H isothiocyanations offer notable environmental benefits due to their efficient atom and step economy, their substrate scope is currently limited to benzyl compounds. Expanding this scope beyond benzylic positions is crucial for the synthesis of diverse and complex isothiocyanates, especially for pharmaceutical purposes. Therefore, developing isothiocyanation methodologies that do not rely on primary amines and prefunctionalization is imperative. In biosynthetic pathways, iron-dependent halogenases facilitate selective chlorination and bromination of aliphatic carbon centers in bioactive natural products. These reactions proceed via a square-pyramidal reactant complex, where the iron(II) cofactor is coordinated by two enzyme histidines, the halide ion, and bidentate 2-oxoglutarate (2OG). Upon capturing O2 at its vacant sixth site, decarboxylation of 2OG to succinate occurs, leading to the formation of a high-valent cis-FeIV(O)(halide) intermediate. This intermediate abstracts a hydrogen atom from the substrate carbon to form a cis-FeIII(OH)(halide) cofactor and an adjacent carbon radical (C•). The reaction concludes with radical coupling of C• with the halogen, reducing the cofactor back



to its Fe(II) state. Halogenases can also catalyze azidation, isocyanation, and nitration in the presence of suitable anions. However, the possibility of isothiocyanation within this reactivity manifold remains uncertain. Previous observations have shown a newly discovered freestanding amino acid halogenase, BesD selectively chlorinating its native substrate L-lysine to produce 4-Cl-L-lysine, and it can also catalyze azidation and bromination with exogenous sources of azide or bromide. Here, we report the first instance of isothiocyanate incorporation into the native substrate L-lysine using BesD halogenase in the presence of an exogenous isothiocyanate source. The formation of the desired product was unequivocally confirmed through liquid-chromatography mass spectrometry (LCMS) and isotope labeling techniques. Importantly, this reaction is not limited to native L-lysine but can also be extended to other substrates such as D-lysine, L-ornithine, and L-homolysine, showcasing its broad substrate scope. This discovery not only opens avenues for non-native organic reactions utilizing halogenase enzymes but also presents an enticing challenge for directed evolutionary processes in biocatalysis and organic catalysis.

15 **Do different bee species modulate their feeding behavior to regulate their protein and lipid macronutrient intake?**

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The decrease in nutritional resources for pollinators, due to intensified land use and habitat loss, poses a great threat to global bee populations. But our understanding of the specific nutritional needs of many bee species, especially for bee species other than honey bees, is still very limited. Pollen is the primary source of protein and lipids for bees, but the nutritional content of pollen varies among different flowering plant species. As a result, bees may have developed the ability to assess pollen quality and adjust their foraging behavior to obtain a balanced nutrition from multiple food sources. To investigate this, we conducted(/ing) a series of four lab-based experiments to assess the ability of two solitary bee species, *Megachile rotundata* and *Osmia cornifrons*, to regulate their intake of protein and lipids. Newly emerged one-day old female bees were fed ad libitum individually with six different synthetic diets (in 30% w/w sucrose solution) that varied in their protein-to-lipid ratios (P:L) - protein only, 25:1, 10:1, 5:1, 1:1 and 1:10 P:L, and followed their survival and food consumption. The leafcutting bees, *M. rotundata* preferentially consumed diets ranged from 10:1 to 1:1 P:L, thus prioritizing protein consumption. But, when they had high-fat, low-protein diets, they overconsumed lipids in order to meet their protein intake target. This behavior suggests a regulatory mechanism in which the bees adjust their intake to prioritize protein and balance their protein:fat intake. *M. rotundata* bees had best survival rates in diets with 10:1 and 5:1 P:L ratios, with the risk of death increasing when the P:L ratio dropped below 5:1. We are currently analyzing the dietary preferences of *O. cornifrons*. Understanding the specific macronutrient requirements and regulation in bees is crucial for comprehending their foraging behavior in the field and for explaining the patterns of host-plant species choice among solitary bees, enabling the creation of habitats that are nutritionally and ecologically beneficial for a diverse community of pollinators. Our study also provides insights to

improve the development of supplementary foods and beekeeping nutritional practices for managed solitary bees.

16 **A new frontier in SARS-CoV-2 therapy: Therapeutic Interfering Particles (TIPs) demonstrate potent replication inhibition of the virus**

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The ongoing evolution of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), characterized by the emergence of variants and escape mutants, poses significant challenges to existing treatments. To address this issue, we investigated a therapeutic approach utilizing Defective Viral Genomes (DVGs), known for their ability to outcompete and parasitize the parent viruses. We developed Therapeutic Interfering Particles (TIPs) based on DVGs using specific non-protein-coding segments from the SARS-CoV-2 genome, comprising approximately 10% of the total genome. Our study evaluated the therapeutic potential of these TIPs in SARS-CoV-2-infected cell cultures and mice. Transfection of TIPs into Angiotensin-converting enzyme 2 (ACE2)-expressing human lung cell cultures infected with SARS-CoV-2 (B.1 lineage or ancestral strain) resulted in a significant reduction in viral replication, exceeding 100-fold (128-fold by qPCR, >6 Logs by TCID50). Similar reductions in virus titer were observed in cell cultures infected with SARS-CoV-2 variants Delta and Omicron BA.1. Furthermore, when tested as a therapeutic intervention in ACE2-mice infected with SARS-CoV-2 Omicron BA.1, the TIPs demonstrated the ability to significantly reduce lung viral titers by over 20-fold (assessed by qPCR and TCID50). Our findings highlight the potential of TIPs as promising treatment options against SARS-CoV-2, underscoring the necessity for further investigations to optimize dosing and timing.

Poster Presentations

Ecology, Agriculture and Plant Biology

17 **Hare Today, Gone Tomorrow: Recent Snowshoe Hare Range Contraction in Pennsylvania**

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The range of the snowshoe hare (*Lepus americanus*) has been contracting northward along its southern boundary over the last century. In Pennsylvania, winter temperatures and snowfall have been identified as key drivers of this range shift; changes in the distribution of hunter harvests in the late twentieth century show a northward contraction to the coldest regions of the state, and spatial variation in snowfall has been found to explain snowshoe hare site occupancy in these areas. In 2023, we used occupancy modeling based on presence-absence data from tracks and fecal DNA to model current snowshoe hare occupancy across northern Pennsylvania,

with a goal of assessing whether range contraction had continued as predicted based on occupancy data collected in 2004. The best model indicated that winter snowfall remained an important factor explaining snowshoe hare occupancy, along with the amount of surrounding forest cover and the proportion of that forest cover in the early-successional stage. Using this model to estimate snowshoe hare occupancy probabilities across the study area in 2004 and 2023 showed an overall decline in occupancy, with the total area with a ≥60% predicted probability of occupancy declining by 79%. This contraction is occurring faster than was previously predicted and was not mitigated by increases in the amount of early-successional forest cover in the study area over the last two decades. These findings have negative implications for the persistence of snowshoe hares in Pennsylvania as the decreasing trend in winter snowfall is expected to continue.

18 **Beyond Pigments: A Microbial Tale of Flavonoid-Induced Phyllosphere Changes in Maize**

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The term phyllosphere refers to the ecosystem on the surface of plant leaves, hosting various microorganisms like bacteria and fungi. This micro-world plays a crucial role in the interactions between plants and their surroundings. It affects plant health, disease resistance, and the overall ecosystem. Understanding the phyllosphere is key to comprehending how plants interact with microbes, which has implications for sustainable agriculture and ecosystem well-being. Flavonoids are a group of plant secondary metabolites which exert multifaceted effects on plants, shielding plants from pests, UV-induced oxidative damage, and acting as reactive oxygen species (ROS) quenchers. Additionally, flavonoids influence plant interactions with microbes due to their antibacterial and antifungal properties. Recent studies in Chopra lab, (Plant Science, PSU) have found higher mortality rates among fall armyworm (FAW) larvae when consuming leaves from maize rich in flavonoids, in contrast to standard maize. This discovery prompted an exploration into the underlying mechanisms, which eventually led to the preliminary results of identification of distinct variations in the microbial populations present within the regurgitant and midguts of fall armyworms fed leaves of flavonoid-rich maize compared to those fed non-flavonoid maize. This finding raises an intriguing hypothesis: could the high levels of flavonoid compounds in the leaves of these maize lines be altering the microbial communities on the leaf surfaces? Additionally, if the flavonoids are altering the microbial communities, are the alterations responsible for the observed increase in mortality of the fall armyworms? This project aims to decipher the intricate relationship between endogenous flavonoids in maize and the dynamic interaction with the phyllosphere microbial and FAW gut communities. Utilizing bacterial 16S and fungal ITS sequencing, we are identifying unique microbes associated with flavonoid and non-flavonoid producing maize leaves, as well as the guts of FAW which feed on those leaves. We are also trying to understand the role of high sugar content in modulating these phyllosphere communities. This research will provide valuable insight for the future development of high-flavonoid and high biomass/carbohydrates varieties of maize that can be beneficial as food, feed, and fiber sources, all while maintaining natural pest resistance that

reduces the need for pesticide applications.

19 **Thermopriming mitigates the effects of heat stress by modulating the expression of Heat shock factors in *Brassica juncea* (Indian mustard)**

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Brassica juncea (also known as Indian mustard) is an important vegetable oil seed crop whose growth and productivity is severely affected by heat stress. Interestingly, pre-exposure to sublethal heat stress, referred to as thermopriming, can remarkably enhance the plant heat stress tolerance. However, little is known about the impact of thermopriming on heat stress response of the important oil seed crop, *B. juncea*. In this study, we investigated the basal and thermopriming-induced heat stress response of 16 different cultivated, agronomically important varieties of *Brassica juncea*. Based on their basal heat stress response, we classify the varieties as heat sensitive, moderately tolerant or tolerant. Notably, almost all the varieties displayed enhanced heat stress tolerance upon thermopriming (acquired thermotolerance), albeit to varying magnitudes. Strikingly, the high oil-yielding, drought tolerant, heat sensitive variety Pusa Bold showed remarkable acquired thermotolerance upon thermopriming. Investigations in Pusa Bold indicate that the thermopriming-induced acquired thermotolerance predominantly correlates with the rapid activation of ROS scavenging mechanisms and differential expression of Heat Shock Family of transcription factors (BjHSFs). Taken together, our study reveals the positive impact of thermopriming in alleviating the harmful effects of heat stress on *Brassica juncea* seedlings and paves the path to enhance heat stress tolerance for improved productivity in the important oilseed crop Indian mustard.

20 **Assessing the significance of genotype versus nitrogen treatment on facilitation of nitrogen fixation by Latin American common bean (*Phaseolus vulgaris* L.)**

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The Latin American common bean (*Phaseolus vulgaris* L.) is a legume widely considered to be a poor facilitator of nitrogen fixation. This occurs in specialized root organs called nodules that host endosymbiotic bacteria known as rhizobia. Current farming practices for common bean rely heavily on anthropogenic nitrogen fertilizers, which are costly and negatively impact the environment. In addition, heavy artificial selection present in cultivar lineages of beans severely reduces genetic diversity in populations of commercially available beans. As an ancient crop, common bean has been subject to both artificial selection and genetic bottlenecks as a result of domestication. This has gradually reduced genetic diversity within different populations over time. As the formation of legume-rhizobia endosymbiosis is largely dependent on the host legume's gene expression, the reduced ability to facilitate nitrogen fixation may be explained by the loss of genetic variation in common bean over time. However, soil nitrogen presence, such as

anthropogenic nitrogen fertilizers, can also inhibit nitrogen fixation. We hypothesized that genotype would have a more significant impact on plant ability to facilitate nitrogen fixation than nitrogen fertilization. We measured the quality of nitrogen fixation via proxy measurements like root:shoot ratio, nodule count, and chlorophyll content index (CCI) to determine the statistical significance of nitrogen treatment group versus bean genotype. Despite a small sample size, our data suggest that genotype is a more significant indicator than nitrogen fertilization in determining how much nitrogen fixation can be facilitated by common bean. These findings further emphasize the importance of genetic variation within populations of common bean crops and the potential to reduce nitrogen fertilizer usage on crops of common bean via selecting for better facilitation of nitrogen fixation.

21 **Forest Management and Climate Change in the Eastern United States: A Women's Perspective**

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Department of Ecosystem Science and Management

Women have the potential to be a major contributor to climate change solutions through forest management. In the gender studies literature, however, there is little inquiry into the role women may have on forests and climate change in the United States. Thus, we conducted a qualitative assessment study using interviews to further understand (a) which values about forests do women share, (b) what strategies women are imposing to protect their forest from climate change, (c) how women think they would be impacted by the changes in forest use due to climate change, and (d) what the barriers to women are acting in this space. To build a framework of investigation, initial interviews were conducted with 12 women forest owners within Women Owning Woodlands (WOW) network in Pennsylvania and South Carolina in the spring of 2022. Results showed that women value their forest for many reasons including biodiversity, ecosystem services, family bonding, and recreation. But when it comes to climate change, women appear divided as to the level of threat climate change may have on forests. Women think they would go through varying emotions including from emotional distress to passive acceptance if the forest benefits are changed by climate change. Additionally, women were discovered to go through several barriers (knowledge, language, capital, and trust issues) in forestry. Thus, results suggest a need to focus on outreach and education to help women connect their values to climate change mitigation/adaptation efforts and to empower women to act as forest stewards.

22 **Exploring the dynamic interplay of biotic and abiotic factors in microbial community coalescence**

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Community coalescence is defined as the wholesale mixing of ecological communities. After a coalescence event (e.g., fecal microbiota transplantation, organic amendments in soils), a dynamic interplay of biotic and abiotic factors controls the patterns of community reassembly and stabilization over time. However, we still lack a comprehensive framework to properly

parametrize and study compositional and functional outcomes of community coalescence. Here, we use a soil microcosm experimental system to parametrize the dynamics of microbiota reassembly using time-series analysis. For that, distinct soil types varying in biological and physicochemical properties (i.e., with distinct edaphic and eco-evolutionary histories) were experimentally manipulated using a dilution-to-extinction approach and mixed in pairs. Reassembly of the soil microbial community was tracked over time (i.e., 1, 5, 15, and 30 days) following coalescence. We used a combination of bacterial 16S rRNA high-throughput sequencing, Biolog EcoPlate assays, and a series of enzymatic essays to track temporal shifts in community taxonomy, metabolic profile, and enzymatic activities, respectively. Overall, our results revealed that microbial diversity affects the relative dominance of donor communities on community reassembly and that donor dominance is strongly associated with the presence of stringent abiotic factors in the donors' home environment. Moreover, we detected an overall decrease in metabolic functions following coalescence, despite a slight increase in enzymatic functioning at high-diversity treatments. Collectively, this study provides a new model that partitions the dominance of donor biotic and abiotic factors that determine the compositional and functional outcomes following community coalescence.

23 **An Ethno-Directed Metabolomic Evaluation of the Medicinal Plant Ghost Pipe (*Monotropa uniflora*)**

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Ghost-pipe (*Monotropa uniflora*, Ericaceae) is a widely distributed North American plant that is used in contemporary folk herbalism in the United States. The species also has a rich ethnobotanical history, and has been used as an analgesic, to treat inflammation, and to allay symptoms of emotional distress. Despite this history of use, little is known about phytochemistry, and it is unclear whether it possesses compounds of medicinal or toxicological activity. Additionally, ghost-pipe is myco-heterotrophic, or parasitic to mycorrhizal fungi. Thus, its secondary metabolite profile—and therefore medicinal properties—may be significantly impacted by changes in its host environment. Using the medicinal traditions surrounding ghost pipe as a guide, my project integrates untargeted metabolomics approaches to study the ecological and bioactive chemistry of this plant with bioactivity assays to further understand its medicinal properties and potential toxicity. To better guide my experimental methods, I first launched a digital survey for ghost pipe consumers to document how people today harvest, prepare, consume, and/or prescribe ghost pipe as a medicinal plant—and to what extent. The survey remained open for 14 months and received 607 responses from 42 US states and 6 Canadian provinces. Preliminary results indicate pain relief as the most common use of this plant, followed by anxiety relief and relaxation. The most common mode of preparation reported is a tincture of fresh aerial plant material. Historical records, by contrast, include the use as an anticonvulsant and recommend ingesting a dried powder of the root. However, both my survey and historical sources mention its ability to relieve pain. Informed by these results, in the summer of 2023 I collected ghost pipe samples from 16 sites across four U.S. states and collected a panel of environmental data such as dominant overstory species and soil type for each sample. The plant material was

extracted according to the two most common forms of preparation reported in my survey, and the extracts were then profiled with UHPLC-MS using untargeted metabolomics methods. The resulting metabolomics data will be used to characterize the secondary metabolite profile of ghost pipe through molecular networking. I will also integrate this metabolomic data with the collected environmental data in multivariate statistical models to determine the effect of host environment on its metabolome. With an understanding of the chemical composition of ghost pipe, I will be able to move on to evaluating ghost pipe's therapeutic potential through bioactivity assays and putatively identify the active compounds, laying the foundation to develop ghost pipe as a novel and effective alternative for those in pain. I propose that this ethno-directed study of ghost pipe will both guide me to impactful results most efficiently and ensure that these results are relevant to the public, especially to those people who are already invested in ghost pipe.

24 **Morphological and cellular responses of roots and root hairs of *Arabidopsis thaliana* to changing mechanical environments**

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Plants experience a diverse array of mechanical forces throughout their development and life cycle. These include external forces like gravity, wind, and damage from herbivory, as well as internal forces like turgor pressure, cytoskeletal mechanics, and compression forces at organ junctions. These mechanical stimuli affect plant development at both macroscale and cellular levels partly because of mechanical perception and mechanoresponses mounted by plants. Plant cell walls, which surround and support every plant cell, represent a dynamic mechanical interface between plant cells and the mechanoenvironment. Here, we studied morphological changes in *Arabidopsis thaliana* roots and root hairs in response to changes in the mechanical properties of the growth environment and the functions of cell wall components, including cellulose, hemicellulose, and pectins, in the resulting growth phenotypes. We also examined intracellular calcium signaling in root hairs as a potential signaling nexus for mechano-induced cell wall remodeling. We found that the morphological parameters, such as root length and root hair count, decrease with an increase in the stiffness of the external mechanical environment. We also observed a decrease in the root hair growth rate and an increase in calcium signaling intensity at the root hair tip with increasing mechanical impedance of the growth media. Insights from this research will be helpful in engineering resilient root systems for agricultural crops growing in challenging soil environments.

Chemistry, Biochemistry, and Structural Biology

25 **Reaction is not required to form Turing-like patterns for proteins!**

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Biological systems exhibit a wide variety of pattern formations, and these patterns are often crucial for the proper functioning and development of organisms. Animal coat patterns,

fingerprints, and biofilm formation are some examples for these patterns. Several mechanisms behind the biological pattern formation were identified including reaction-diffusion systems and collective behavior and interaction. Here we demonstrate an enzyme pattern formation phenomenon on the air-liquid interface. A concentration of 50 µM of urease was injected into the 3 ml of glucose or sucrose solution in a petri dish. Then, after sometimes the spiral shape pattern formation was observed. All solutions were prepared in MES buffer at pH 6. Pattern formation occurs when the enzyme suspended in a less dense solution is introduced into the more denser solution. The calibration plots of time versus concentration of the solution in the petri dish were drawn using different concentrations of D-glucose and sucrose solutions and the linear relationship between the time taken to form the pattern and the concentration was observed. The uniqueness of our system is that the reaction-diffusion does not govern the pattern formation. This enzyme pattern formation is not only limited to enzymes, and we were able to observe it even with the fluorescence dye. This process may be a fundamental phenomenon in nature and may be related to the formation and regulation of spatial patterns in nature. This phenomenon may occur combination of fundamental mechanisms and phenomena like Bernard-Marangoni convection, density gradient, and viscosity of the solution.

26 **Microengineering of Collagen Matrices: Enhancing Fibril Alignment via 3D Bioprinting**

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The extracellular matrix (ECM) serves as a significant regulator of cellular behavior *in vivo*, with its biophysical characteristics such as stiffness, porosity, composition, and fiber alignment (anisotropy) notably influencing cell responses. Among ECM constituents, type I collagen (collagen I) stands out as a structural element, offering a versatile hydrogel platform that mirrors *in vivo* mechanical properties. Tissue engineering applications increasingly utilize the potential of aligned collagen fibers to mimic native tissue architectures. Here, we present an approach for developing 3D bioprinted collagen I hydrogels with controlled anisotropic fiber arrangements. This technique holds promise for enhancing the physiological relevance of *in vitro* cell culture models. Aligned collagen fiber architectures not only offer insights into fundamental cell-material interactions but also present opportunities for various biomedical applications such as cornea and cartilage tissue regeneration. In the cornea, aligned collagen fibers ensure transparency and vision clarity, while in cartilage, they provide mechanical strength and flexibility. Disruptions in alignment can lead to tissue abnormalities and functional impairments. The engineered collagen matrices via 3D bioprinting could offer precise customization for personalized therapies, promising innovative solutions for cornea and cartilage tissue repair.

27 **Probing the role of 2',3'-cNMP's inside E. coli**

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Cells regulate their responses to environmental stimuli and stress to allow them to maintain homeostasis and adapt to changing environments. One response system that cells use to respond

to their environment is intracellular signaling molecules, termed second messengers. These signaling molecules help regulate cellular functions by binding to various targets, such as proteins and riboswitches which activate or deactivate different cellular pathways. Key second messengers are based on nucleotides and within bacteria, include 3',5'-cyclic nucleotide monophosphates (3',5'-cNMPs) as well as cyclic-di-nucleotide monophosphates (c-di-NMPs) which help regulate important cell functions such as DNA repair, motility, and virulence. Besides these well-studied molecules a number of less studied nucleotide-based signaling molecules exist, a group of these being, 2',3'-cyclic nucleotide monophosphates (2',3'-cNMPs). 2',3'-cNMPs were discovered in *Escherichia coli* (*E. coli*) over 50 years ago but have not been the focus of major studies in bacteria until recently. Within our group it has already been discovered that 2',3'-cNMPs affect key biological processes such as translation and by understanding these signaling molecules, their targets, and their binding sites, new ways to probe the cell can be developed, providing a powerful tool to further elucidate the cellular pathways they affect and understand their role in important cellular functions.

28 **Nested Biofabrication: Matryoshka-Inspired Intra-Embedded Bioprinting**

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Engineering functional tissues and organs remains a fundamental pursuit in bio-fabrication. However, the accurate constitution of complex shapes and internal anatomical features of specific organs, including their intricate blood vessels and nerves, remains a significant challenge. Inspired by the Matryoshka doll, here a new method called "Intra-Embedded Bioprinting (IEB)" is introduced building upon existing embedded bioprinting methods. a xanthan gum-based material is used which served a dual role as both a bioprintable ink and a support bath, due to its unique shear-thinning and self-healing properties. IEB's capabilities in organ modeling, creating a miniaturized replica of a pancreas using a photocrosslinkable silicone composite is demonstrated. Further, a head phantom and a Matryoshka doll are 3D printed, exemplifying IEB's capability to manufacture intricate, nested structures. Toward the use case of IEB and employing an innovative coupling strategy between extrusion-based and aspiration-assisted bioprinting, a breast tumor model that included a central channel mimicking a blood vessel, with tumor spheroids bioprinted in proximity is developed. Validation using a clinically-available chemotherapeutic drug illustrated its efficacy in reducing the tumor volume via perfusion over time. This method opens a new way of bioprinting enabling the creation of complex-shaped organs with internal anatomical features.

29 **Diverging Mechanisms of FXR Activation by High-Potency Bile Acid Homologues**

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The farnesoid X receptor (FXR) is the main target receptor of drug developers working on treatments for non-alcoholic fatty liver disease. It is widely known that FXR exhibits ligand-dependent activation, but the mechanism of activation or features of ligands that correlate

with their ability to activate FXR remains unclear. My research has identified that bile acids and their homologues which are highly active (transcriptional EC50 < 1 μM) follow one of two activation mechanisms, initiated by either a C6 or C17 substituent, and less activating ligands are unable to initiate activation via either pathway. In addition, one of the identified activation mechanisms is similar to a mechanism previously seen only in non-steroidal ligands, forming a more cohesive picture of FXR activation by tying together two previously unrelated ligand categories.

30 **Heme-oxygenase-like protein, FlcE undergoes a four-electron oxidation to effect multiple turnovers in the absence of any reductant**

Sohini Saha, Xavier Enrique Salas Sola, Mrutyunjay A. Nair, Chi-Yun Lin, Carsten Krebs, J. Martin Bollinger Jr.
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Heme-oxygenase-like diiron oxidases and oxygenases (HDOs) are an emerging superfamily of dioxygen-activating non-heme diiron enzymes. In terms of functional diversity, initially characterized members represented just two functional subclasses: the N-oxygenases and the C-C-bond-fragmenting desaturases. More recently, a burst of functional annotations of new members of the superfamily have rapidly broadened its reactivity profile. One such new HDO, FlcE, which is found in the fluopsin C biosynthetic pathway, catalyzes both N-oxygenation and decarboxylation of (2R)-{[(2R)-2-amino-2-carboxyethyl]sulfanyl}butanedioic acid [(2R)-S-succinyl-L-cysteine)] transforming it to (2R)-{[2-hydroxyimino-ethyl]sulfanyl}butanedioic acid. While reactions with only oxidative decarboxylation or N-oxygenation outcomes have previously been observed for HDOs, both reactions occurring together is unique, thus giving rise to speculation of a novel mechanistic route. FlcE, which lacks the seventh carboxylate ligand that is conserved in the previously characterized HDO N-oxygenases, SznF and RohS, cannot by itself configure to achieve its O2-reactive holoenzyme form. Rather, FlcE is "substrate triggered", meaning that it reacts with dioxygen only in the presence of its substrate, (2R)-S-succinyl-L-cysteine. Upon exposure of the FlcE complex with iron(II) and substrate to dioxygen, a transient, intensely absorbing peroxodiiron(III) intermediate rapidly accumulates, the identity of which has been confirmed by Mössbauer spectroscopy. The kinetics of accumulation and disappearance of the intermediate in reactions with excess substrate and varying molar ratios of O2:enzyme imply that the FlcE reaction is catalytic in the absence of reductant. A similar observation of the intermediate kinetic behavior is also made in reactions with excess O2 and varying substrate:enzyme, thus further corroborating the catalytic behavior of FlcE. Furthermore, quantification of the amount of substrate consumed and product formed by LC-MS in an assay conducted under limiting enzyme and iron(II) conditions in the absence of any external reducing agent demonstrates that FlcE effects multiple turnovers. Given precedent for the four-electron oxidation of an aminoarene to the corresponding nitrosoarene by the peroxodiiron(III) intermediate in the ferritin-like non-heme diiron oxygenase CmlI, the data suggests a pathway for the FlcE reaction in which the four-electron N-oxidized nitroso intermediate undergoes unassisted decarboxylation and O-protonation to the aldoxime final product. According to this mechanism,

in order to arrive at the four-electron N-oxidized nitroso intermediate, the two-electron N-oxidized hydroxyl amine intermediate must undergo a coupled redox reaction with the diferric decay product from the peroxodiiron(III) intermediate, to regenerate the diferrous cofactor. Observation of the reformation of the diferrous quadrupole doublets at a time point corresponding to the decay of the peroxodiiron(III) intermediate in a time-resolved Mössbauer spectroscopic study provides strong proof for a four-electron oxidation pathway for FlcE. A 1:1 stoichiometric evidence of the consumption of substrate and dioxygen by LC-MS fully negates the possibility of a second molecule of dioxygen being involved in a single FlcE turnover, thus affirming the proposed four-electron oxidation mechanism. This study reports the first occurrence of a fully self-reliant multi-turnover protein in the HDO superfamily and elucidates its catalytic mechanism without the aid of an external reducing system.

31 **Quantifying lipid membrane asymmetry by a novel lipid bilayer decoupling assay**

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The asymmetric arrangement of phospholipids between the two leaflets of a cell plasma membrane is an integral part of cellular function. However, the distribution of lipids between the two leaflets of supported lipid bilayers (SLB) has been notoriously difficult to quantify. This is unfortunate as the SLB platform has served for decades as a valuable model system for elucidating the biophysics of cellular membranes. Due to the ultra-thin nature of a lipid bilayer (i.e., approximately 4.5 nm thick), there are few techniques available that can accurately distinguish the chemistry of the two individual leaflets. In an effort to remedy this problem, we have created a bilayer unzipping assay as a promising technique to study lipid asymmetry. By forming a substrate-membrane-substrate sandwich, we have found that the two leaflets of an SLB can be decoupled into two individual monolayers that can be separately subjected to a variety of bioanalytical characterization assays. Our results show that the distribution of lipids in the two leaflets is significantly different than in the vesicles from which they are formed. Overall, we find that lipid-substrate interactions are the prominent driving force behind this difference.

32 **Engineering Sustainable Biopolymers made from bio-renewable Resources for Additive Manufacturing**

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Additive manufacturing techniques like stereolithography (SLA) 3D printing enable the production of complex, high-resolution structures. However, the petroleum-derived resins typically used often limit the mechanical performance. This study pioneers the development of bio-based polyester resins from bio-renewable sources optimized for enhanced mechanical properties in SLA printing applications. The polyester resins were synthesized via polycondensation of bio-based diacids (itaconic and succinic acid from hydrolysis of glucose extracted from biomass) and diols (1,2-propanediol, 1,4-butanediol, and 1,8-octanediol from fermentation of crop waste). Utilizing annually renewable feedstocks rather than finite fossil fuels increases sustainability while

providing opportunities to diversify chemical structures and tailor resin properties. Varying the bio-based diacid and diol components enabled tuning the molecular weights, crosslink densities, rheological behavior, and mechanical characteristics. Shear-thinning rheology ideal for SLA was achieved. Tensile testing revealed tunable strengths from 0.2-2.0 GPa and 1.7-7.5% elongations by the molecular design of the bio-based polyesters. 3D printing trials demonstrated their capabilities for high-resolution SLA printing using 405 nm UV curing. The tailored bio-based polyester resins can be leveraged for sustainable additive manufacturing of varying-strength objects like biomedical implants for tissue engineering. This renewable material solution enables high-performance SLA 3D printed products, reducing reliance on petroleum-based and environmentally detrimental materials. Designing resins from bio-derived building blocks expands opportunities for sustainable and circular manufacturing.

Genetics and Molecular Biology

33 **cGAS-STING pathway in Stress Erythropoiesis**

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Significance and purpose of the study: Anemia of inflammation (AI) is a widespread blood disorder affecting billions of people globally. Current treatment options, such as recombinant erythropoietin therapy (rhEpo) and blood transfusions, have limitations including alloimmunization and limited efficacy in AI cases. To develop alternative treatment strategies, it is crucial to understand how the body recovers from anemic stress. Under normal conditions, steady state erythropoiesis constantly generates new red blood cells in the bone marrow to maintain tissue oxygenation. However, during pathological conditions like infection or tissue damage, stress erythropoiesis becomes the primary pathway for erythrocyte production. Inflammation shifts hematopoiesis towards myelopoiesis, reducing erythroid output. Stress erythropoiesis, involving stress erythroid progenitors (SEPs), ensures erythroid homeostasis by generating a bolus of erythrocytes in the spleen. Unlike steady state erythropoiesis, in stress erythropoiesis the pro-inflammatory signals drive the proliferation of immature cells and inhibit their differentiation. Understanding the cues that prevent differentiation and facilitate rapid expansion of SEPs is crucial for this process. Exploring the regulation of stress erythropoiesis opens new possibilities for treating anemia. The primary objective of this study is to investigate the role of the cGAS-STING pathway in stress erythropoiesis and its regulation of the inflammatory metabolic environment. cGAS (cyclic GMP-AMP synthase) is an enzyme that recognizes cytoplasmic DNA and activates the STING (Stimulator of Interferon Genes) pathway, leading to the production of interferons and inflammatory responses. By studying this pathway, we can gain insights into the involvement of Damage Associated Molecular Patterns (DAMPs) in stress erythropoiesis and identify potential treatments for alleviating Anemia of Inflammation. The cGAS-STING pathway plays a critical role in recognizing non-genomic DNA within cells and initiating the innate immune response. Dysregulation of this pathway can lead to disruptions in cellular and systemic immunity, contributing to inflammatory and auto inflammatory diseases.

Therefore, understanding the cGAS-STING pathway's involvement in stress erythropoiesis can advance our understanding of the field and pave the way for new therapeutic approaches.
Research Methodology: The murine model is widely used in the study of stress erythropoiesis due to its physiological similarity to humans. Paulson lab has established an in vitro culture system that mimics the *in vivo* environment and allows for the investigation of stress erythropoiesis. In this system, unfractionated murine bone marrow cells are cultured in amplification media (SEEM) for 5 days, followed by transfer to differentiation media (SEDM) which is SEEM supplemented with erythropoietin and kept in low oxygen conditions (1% O2) for 3 days. This triggers the differentiation of the cells into BFU-es, the first committed precursors for differentiation. In the *in vivo* model, inflammatory anemia is induced in mice using a sterile inflammation method by injecting the mice with Heat Killed *Brucella arbortus* (HKBA). For this study, both STING knockout mice (KO) and wild type (WT) C57BL/6 mice are being utilized.
Central Hypothesis: The activation of the cGAS-STING pathway triggers the Interferon type 1 signaling, which in turn regulates the initial stages of Stress Erythropoiesis.
Aim1: To characterize the role of STING in Stress Erythropoiesis: Preliminary data demonstrates that mice lacking the STING gene (STING KO) exhibit a noticeable impairment in stress erythropoiesis. In *in vitro* studies, STING KO mice exhibit reduced growth in SEEM culture. Additionally, SEPs derived from STING KO cultures display diminished differentiation compared to wild-type (WT) on SEEM day 5, as observed through BFU-E culturing. In *in vivo* experiments, when sterile inflammation was used to induce anemia of inflammation, STING KO mice exhibit a clear defect in anemia recovery compared to WT mice. The collected data strongly suggest that the absence or dysfunction of the STING gene leads to a distinct phenotype in stress erythropoiesis. These findings highlight the crucial role of STING in the regulation of stress erythropoiesis and its significance in maintaining erythroid homeostasis. To gain a deeper understanding of the involvement of the STING pathway in stress erythropoiesis, I propose conducting an RNA-seq analysis to identify the targets of this pathway within stress erythroid progenitors (SEPs). Additionally, considering the observed disparities in metabolic environments during the expansion and differentiation of SEPs, I intend to assess the metabolic profile of STING knockout (KO) mice to investigate potential metabolic defects associated with the absence of STING. These investigations will provide valuable insights into the molecular and metabolic mechanisms regulated by STING in stress erythropoiesis and contribute to our using cGAS knockout (KO) mice to further explore the pathway and gather additional information.
Aim2: Characterize the type I IFN response pathway in Stress Erythropoiesis.
Method: To investigate the role of the Type 1 interferon (IFN) pathway in stress erythropoiesis (SE), we will conduct several experiments. This includes examining the expression levels of Type 1 IFNs (IFN alpha and beta) and Interferon Regulatory Factors (IRF3 and IRF7) using qPCR to identify any differential expression between wild type (WT) and STING knockout (KO) cells. We will also employ Interferon alpha/beta receptor (IFNAR) knockout (KO) mice to assess the significance of the Type 1 IFN pathway in SE by measuring hematocrit levels, conducting flow cytometric analysis of stress erythroid progenitors (SEPs), and performing RNA-seq to identify targeted cellular pathways. Additionally, we will explore the impact of the microenvironment on SEP development by employing flow cytometry to examine cells within the niche and determine if the lack of Type

1 IFN signaling disrupts SEP development.

34 **Adenylosuccinate alleviates mobility deficits associated with Adenylosuccinate Synthetase deficiency in *Caenorhabditis elegans***

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Inborn errors of purine metabolism have been linked to various neurological and muscular disorders that are under-reported and often go undiagnosed or misdiagnosed because their symptoms may often mimic other, more detectable, diseases. The links between purine metabolism and the mechanisms of these diseases remain understudied and effective therapies are unavailable due to our insufficient understanding of them. Therefore, these diseases are of major clinical significance and pose some of the most challenging enigmas in medical diagnosis and treatment. ADSSL1-Myopathy is an ultra-rare muscular disorder caused by a mutation in the purine nucleotide cycle (PNC) gene, *ADSSL1*. *ADSSL1* encodes adenylosuccinate synthetase, an enzyme in the PNC that is important for energy metabolism in the muscle. Some of the symptoms of the disorder include movement dysfunction, muscle weakness, lipid accumulation in the muscles, and the disruption of proper muscle structure. Like other inborn errors of purine metabolism, ADSSL1-Myopathy has been hard to characterize on a molecular level due to a lack of adequate models for its study. We are investigating the biological functions of ADSS using the nematode, *Caenorhabditis elegans*. We have established that *adss-1* RNAi knockdown animals have slower crawling speed, reduced wavelength, reduced thrashing rate, and uncoordinated movement. We have probed the neuromuscular junction through paralysis assays using aldicarb and levamisole but observed no obvious impairments. We have also found evidence of changes in muscle structure and an accumulation and enlargement of lipid droplets in adss-1 knockdown animals. Additionally, we hypothesize that the supplementation of PNC substrates will ameliorate phenotypes associated with the lowered expression of *adss-1* and can be used as a potential therapeutic strategy. We have found that the supplementation of adenylosuccinic acid (ASA) rescues movement phenotypes of speed and wavelength and are continuing to analyze other parameters of movement. This supports the use of other PNC substrates as a therapeutic strategy for the disorder.

35 **Identifying the Mechanism of ATF4 Expression in Stress Erythroid Progenitors**

Sara Trimidal, Robert Paulson
Department of Biochemistry and Molecular Biology

Inflammation impairs steady-state erythropoiesis in the bone marrow and shifts hematopoiesis towards myelopoiesis. Stress erythropoiesis (SE) is a compensatory pathway that increases erythrocyte production to allow the body to recover from the loss of red blood cells. Previous research identified a nitric oxide (NO)-dependent metabolic regulation during stress erythroid progenitor (SEP) proliferation. NO promotes glycolysis and the anabolic serine/glycine and pentose phosphate pathways, thus driving nucleotide and amino acid biosynthesis. The transcription factor ATF4, known to promote expression of genes important in amino acid

biosynthesis and transport, increases in production during SEP proliferation, and ATF4 mutant mice exhibited anemia. We hypothesize that an increase in purines from NO signaling will activate mTORC1 and increase translation of ATF4, which will regulate essential genes that contribute to rapid SEP proliferation. However, our data suggests that ATF4 is translated through the integrated stress response (ISR) pathway with the kinase GCN2, and its translation is not controlled by NO signaling.

36 **Brain organoid models to investigate neurodevelopmental disorders**

Serena Noss, Jiawan Sun, Santhosh Girirajan
Department of Biochemistry and Molecular Biology

Understanding how genetic factors contribute to neurodevelopmental diseases is challenging due to complex gene interactions and highly variable clinical phenotypes. The 16p12.1 microdeletion is a rare copy number variant (CNV) associated with a wide range of neurodevelopmental disorders (NDDs), including autism spectrum disorder, schizophrenia, epilepsy, and developmental delay. This study investigates cellular phenotypes caused by the 16p12.1 deletion and how they are modulated by the specific genetic backgrounds of patients. Induced pluripotent stem cells (iPSCs) were obtained from 16p12.1 patients and their families, as well as a nondeletion control line and a deletion control line generated using CRISPR/Cas9 system. Both 2D and 3D cell culture models were utilized, relying on dual SMAD inhibition to generate neural progenitor cells (NPCs) and mature neurons, as well as cortical organoids in suspension culture. These models were used to investigate cellular phenotypes associated with the deletion, including markers of cell fate during neural differentiation, axis patterning, and proliferation and apoptosis. RNA-sequencing was done on the 2D cultured cells at the iPSC, NPC, immature neuron, and mature neuron stages and the data was analyzed for differentially expressed genes (DEGs) between deletion carriers, noncarriers, and controls. The RNA-seq results indicate several deletion carriers as well as the CRISPR generated deletion have an overexpression of a ventral marker, NKX2.1, as well as an overexpression of GABAergic neuron markers. Immunostaining on mature neurons and three month old cortical organoids also supports these results. These findings suggest an overabundance of inhibitory neurons is a possible mechanism by which the 16p12.1 deletion increases NDD risk; although more work is needed to better understand how additional genetic variants in patient's genetic backgrounds create variation in phenotypes.

37 **m6A-modified RNA in melanoma-derived extracellular vesicles enhances metastasis**

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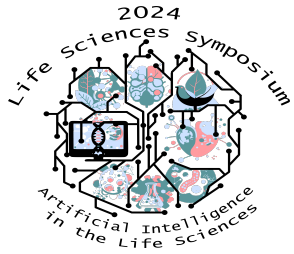
Background: Extracellular vesicles (EVs) contain proteins, nucleic acids, and metabolites within a lipid bilayer. They serve as important vehicles for intercellular communication. Most cell types secrete EVs, but during cancer progression, the EV cargo changes and quantity of EVs released considerably increases. These tumor-derived EVs not only affect their immediate tumor microenvironment but also enter the bloodstream to alter distant tissues, helping to establish a

supportive microenvironment for migrating tumor cell engraftment and outgrowth known as the pre-metastatic niche (PMN). However, the mechanisms for EV-mediated promotion of metastasis remain poorly understood. During our evaluation of patient sera, we discovered that serum RNA from melanoma patients was significantly enriched (p-value < 0.01) for the N6-methyladenosine (m6A) RNA modification compared to non-cancer patient sera. m6A is the most common internal RNA modification and is known to influence splicing, stability, localization, and translation of RNA. It frequently becomes dysregulated in several cancer types, often leading to the promotion of tumorigenesis and metastasis. Our study seeks to determine whether the elevated m6A-RNA in melanoma EVs is functionally important for the EV-mediated acceleration of metastasis. Methods: EVs were isolated from mouse and human melanoma cell lines using differential centrifugation. Nucleoside mass spectrometry was used to quantify m6A. Using genetic, pharmacologic, and transfection-based techniques, m6A levels were altered in melanoma EVs. These EVs were used to determine the ability of EV m6A to activate fibroblasts to a more cancer-associated fibroblast-like state in transwell invasion assays. Additionally, these EVs were injected into syngeneic, immunocompetent mice to assess the effect of EV m6A on PMN formation and melanoma metastasis. Results: We found that reductions in EV m6A decreased melanoma EV-induced effects on recipient cells in culture, inhibited PMN formation in mouse lung tissue, and suppressed melanoma metastasis to mouse lungs. Moreover, this phenotype was reversed by the re-introduction of m6A-enriched but not unmethylated mRNA, enhancing fibroblast invasion and melanoma metastasis beyond control EVs even when non-oncogenic RNA was used. Conclusions: These results suggest that EV m6A aids in fibroblast activation, supporting PMN development and metastasis in melanoma progression. The fact that “neutral” m6A-modified RNA was able to recover loss of endogenous melanoma EV m6A indicates that the m6A itself may be serving as a signal within the recipient cell. Studies are currently ongoing to determine this EV m6A-responsive signaling pathway. Further elucidation of this pathway could lead to the development of epitranscriptomic-modifying drugs as anti-metastatic agents for melanoma patients.

38 **Genetic interactions modulating cellular outcomes contribute to the phenotypic variability in neurodevelopmental disorders**

Jiawan Sun, Serena Noss, Francisca Canzar, Maitreya Das, Deepro Banerjee, Corrine Smolen, Hema Bhavana, Joseph Mao, Anisha Prabhu, Santhosh Girirajan

One of the biggest challenges remaining in elucidating the mechanisms of neurodevelopmental disorders lies in the heterogeneous clinical manifestations, such as incomplete penetrance and variable expressivity, despite affected individuals carrying the same genetic drivers. Copy number variants (CNVs), deletions or duplications of chromosome segments, alter multiple gene dosages and have been associated with complex disorders, including autism, schizophrenia, intellectual disability (ID), and epilepsy. Among these CNVs, 16p12.1 deletion, a 520-kbp deletion, serves as a paradigm to study phenotype variability in complex disorders. Notably, the affected children exhibit a broader range of clinical manifestations compared to their affected parents and demonstrate a higher prevalence of additional rare variants in their genome background. Thus,



we proposed the “two-hit” model. We hypothesized that the genetic interactions between the 16p12.1 deletion (first hit) and other genes (second hits) affected by these additional rare variants give rise to the phenotype variability. To test this hypothesis, we performed RNA-seq on induced pluripotent stem cell lines (iPSCs), neural progenitor cells (NPCs), immature neurons and mature neurons derived from 12 individuals from 16p12.1 deletion patient cohort with neurodevelopmental and psychiatry features, as well as two healthy donors. We also generated a CRISPR/Cas9-mediated deletion line in healthy genome background. To further evaluate the effects on cellular function, we performed genetic marker characterizations, proliferation and apoptosis assays on NPCs and neurons. As a result, shared and distinct differentially expressed genes (DEGs) were identified among families between carrier lines and noncarrier lines, including known risk genes such as FOXG1, CNTN6, and KCNQ5. we also found family-specific transcriptional signatures and cell-type specific effects caused by dysregulations on multiple pathways such as Wnt signaling pathways, Notch signaling pathways and ERK/MAPK signaling pathway, which contributes to the cellular and developmental defects such as excitatory/inhibitory imbalance and hyperproliferation of NPCs in distinct family genome backgrounds. Furthermore, we performed Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) on iPSCs and NPCs, as well as whole genome sequencing (WGS) on all the individuals. We also rescued the dosage of genes within 16p12.1 deletion via the CRISPR activation system delivered by lentivirus to perturb the genetic interactions. By integrating multi-omics with functional characterization, we aim to identify the potential “second-hit” genes and their neuronal functions affected by either coding or non-coding variants through gene regulation. Overall, our study provides important insights into how genetic interactions modulate phenotypic outcomes, which may lead to pathways and target in personalized medicine and therapies of complex disorders.

39 **Using X-ray Histotomography: A Systematic Approach to Cellular Phenotyping Across Animal Models**

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Cellular phenotypes, reflecting genetic and environmental influences, undergo discernible changes in response to anatomical malformations or acquired pathologies like cancer or inflammatory disorders. Understanding these changes is crucial for defining clinically significant phenotypes for histopathological diagnosis. However, the challenge lies in systematically characterizing the vast array of cell types across model organisms. To address this, we propose to quantitatively derive characterizations of morphological variations in normal cells and tissues based on mathematical parameters that capture the relationships between distinct cell types (i.e., gut epithelial cells and fat cells). These parameters, including volume, shape, and principal axes measurements, will provide a more comprehensive understanding of the intricate cell-cell relationships and functional dynamics within each critical cell population. While conventional histological examination remains the gold standard for tissue diagnosis, it is limited in providing

comprehensive three-dimensional visualization and quantitative bioimage analysis of cells and tissues in their volumetric context. To overcome these limitations, we utilize X-ray microtomography (microCT) as a non-destructive imaging method for centimeter-scale organisms. Specifically, our lab optimized a microCT-based histological approach, termed histotomography, which combines histology-level resolution and 3D microscopy imaging to detect micron-scale morphological and cellular features, as well as changes or abnormalities in these features, that are indicative of disease pathology. Our approach, demonstrated in water flea and zebrafish models, now extends to Mexican axolotl larvae. By examining shared features across these aquatic models, we aim to define normal cellular phenotypic variations to develop a machine learning-based algorithm capable of automated recognition of a given cell type despite differences in cell size, tissue morphology, or biological variability among the samples imaged. The envisioned outcome of this research will yield computationally derived characterizations of normal cell morphology across model organisms. This will provide a reference for identifying disease-related alterations in cell structure and function, ultimately aiding in more efficient and accurate disease modeling and diagnosis. This work further serves to expand our laboratory's objective of unbiased, whole-organism computational phenotyping while also providing a greater understanding of genotype-phenotype associations and their clinical implications across model systems.

Immunology and Infectious Disease

40 **Identifying Novel Signaling Mechanisms the Regulate the Development of the Stress Erythropoiesis Niche**

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Steady-state erythropoiesis is a homeostatic process that constantly generates new erythrocytes in the bone marrow to replace senescent erythrocytes removed by the spleen. Inflammation due to infection or tissue damage skews hematopoiesis towards myelopoiesis and inhibits this process. An extramedullary pathway called stress erythropoiesis maintains erythroid homeostasis in such situations and prevents potentially fatal anemias. Stress erythropoiesis uses stress erythroid progenitors (SEPs) to produce a bolus of erythrocytes in periodic waves. These SEPs require a specialized monocyte-macrophage microenvironment called the stress erythropoiesis niche to develop into mature erythrocytes. This stress erythropoiesis (SE) niche develops only in concert with SEPs. The overall objective of my project is to identify novel mechanisms that regulate the development of this niche following acute inflammation. Preliminary work by our lab supports a model wherein the SE niche develops in three stages: First, pro-inflammatory signals increase to recruit monocytes into the spleen and form the early stress erythropoiesis niche. Second, pro-inflammatory signals decrease to terminate monocyte recruitment and establish a niche that supports SEP proliferation. And third, pro-resolving signals increase and establish a niche that promotes SEP differentiation. Previously published work has shown that during infections: i) STAT1 increases the expression of pro-inflammatory cytokines to initiate monocyte

recruitment, ii) nitric oxide decreases the expression of pro-inflammatory cytokines to terminate monocyte recruitment, and iii) erythrophagocytosis increases SpiC expression, which then increases the expression of pro-resolving signals (like GDF15) and promotes SEP proliferation. Main Hypothesis: During anemic stress, tissue-resident macrophages increase their expression of pro-inflammatory cytokines via STAT1-dependent signaling to recruit monocytes into the spleen and form the early niche. As more monocytes get mobilized into the niche, the production of nitric oxide increases, which behaves as a quorum-sensing molecule and decreases the expression of pro-inflammatory cytokines and chemokines to terminate monocyte recruitment. Increased erythrophagocytosis induced by these pro-inflammatory signals also leads to increased expression of SpiC, which finally regulates the expression of pro-resolving cytokines (like GDF15) to establish a niche that supports SEP proliferation.

41 **Investigation of Immunomodulatory Effects of Protein-L for Antibody Engineering**

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Immunotherapy has emerged as a promising approach for treating various malignancies, with therapeutic antibodies playing a pivotal role. However, challenges persist in enhancing their efficacy, particularly in targeting multiple antigens simultaneously to avoid antigen escape and utilizing antibodies as cargo delivery vehicles. To address this gap, we explored the utilization of Protein-L, a protein fragment isolated from bacteria *Peptostreptococcus magnus*, known for its binding affinity to kappa light chains of certain mammalian immunoglobulins, including humans. Protein-L offers unique attributes, among which is its ability to bind to antibodies without interfering with their antigen-binding (Fab) region or stalk (Fc) region, making it an attractive candidate for improving antibody-based therapies. Screening of CD20 and CD3 kappa light chain containing monoclonal antibodies (mAbs: OKT3, HIT3a, 2H7 etc.,) revealed preferential binding of Protein-L on certain clones in our cell-based assays using multi-color flow cytometry. Protein-L mediated higher affinity to 2H7 but not OKT3 and HIT3a. We selected a human CD20 antibody 2H7 clone for proof of concept. Conjugation of biotinylated Protein-L with 2H7 mAb resulted in a 2H7-Ab-PL conjugate. Treatment of lymphoid cancer cell lines, Ramos (B cells, CD20+ CD3-) and Jurkat (T cells, CD3+ CD20-), with 2H7-Ab-PL, revealed unaltered binding specificity to CD20 antigen on Ramos cells. Additionally, the conjugate-induced cytotoxicity is similar to the parental 2H7 mAb. Protein L has been known to downregulate B cell receptor (BCR: surface IgG, IgM) due to its affinity to kappa chains on BCR. Considering free Protein-L's superantigen properties, we conducted cell proliferation assays and calcium flux assays evaluating the immune cell secondary messenger, inositol trisphosphate (IP). Unlike free Protein-L, 2H7-Ab-PL did not elicit super antigenic related proliferation or cell death, likely attesting to their safety and specificity in therapeutic applications. Further, we analyzed immune cell signaling in response to cell surface immunoglobulin modulation/ BCR ligation using anti-human IgG/IgM F(ab)2. Pretreatment of B cells with 2H7 or 2H7-Ab-PL, mediated normal BCR ligation induced calcium mobilization, evidenced by a distinct peak signal from Fluo3 and Fura Red calcium indicator dyes via flow cytometry. In contrast, Protein-L in its free form resulted in delayed time to peak indicative of BCR

downmodulation. Biotin attached to Protein-L serves as a handle for ligating other biomolecules without affecting Fab and Fc function of antibody. Ongoing studies are aimed at functional use of Protein-L for additional payload delivery and designing heterobifunctional paratopes to aim epitopes of interest. Overall, our research endeavors to leverage Protein-L as a versatile handle, enabling the simultaneous engagement of target and effector cells to enhance immune responses and improve therapeutic outcomes and would serve to deliver the cargo. By harnessing the unique properties of Protein-L, our study contributes to advancing the efficacy and precision of antibody-based immunotherapy, offering promising prospects for the treatment of various malignancies.

42 **Dissecting the role of host selenoproteins in the pathogenesis of tularemia**

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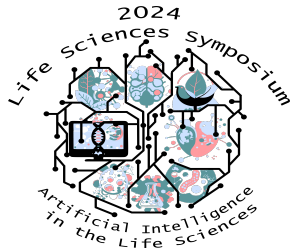
Selenium (Se) is an essential trace element incorporated as amino acid selenocysteine. Selenoproteins, a group of proteins that contain one or more selenocysteine, play a crucial role in the functioning of the brain, thyroid, and male reproduction. Selenoproteins are also key in the optimal functioning of the immune system. There are 25 selenoproteins in humans and 24 is mice. While the majority of selenoproteins are known to have role in regulating the redox status of cells, functions of many selenoproteins are not known. Interestingly, both Se and selenoproteins aid in protection against bacterial infections as several studies have shown a poorer prognosis of patients with lowered serum Se levels in sepsis and high doses of Se as part of adjunct therapy is beneficial. However, the mechanistic basis for this beneficial effect of Se is not known. To establish the specific effect of host selenoproteins during a bacterial infection, we investigated the interplay between the host and *Francisella tularensis* (Ft). Previous studies in our lab have shown that Ft genome does not contain selenocysteine synthesis machinery and the growth and virulence of Ft is not affected by Se status of the growth medium. Interestingly, mice on a selenium-deficient diet succumbed to Ft infection at a higher rate than those on a Se-supplemented diet. Additionally, a macrophage-specific selenoprotein deficient mice (Trspfl/flLysMCre) infected with Ft were more susceptible to Ft infection than the wild-type mice indicating a protective role for one or more macrophage-specific selenoproteins against Ft. In fact, macrophage derived from mice harbored significantly higher number of Ft than the wild-type macrophages suggesting that selenoproteins are critical in restricting intracellular growth of Ft. An RNA-sequencing analysis of Ft-infected macrophages revealed differential regulation of six selenoproteins amongst which was a protein of unknown function namely Selenoprotein W (SelenoW). SelenoW is a 9 kDa protein annotated to have an antioxidant function which has been associated with inflammation and regulation of other selenoproteins. Interestingly, SelenoW deficient mice recovered faster after intranasal infection with Ft and had lower bacterial burden in the systemic organs than the wild-type mice. Consistent to these *in vivo* results, SelenoW deficient bone marrow derived macrophages harbored fewer bacteria than the wild-type macrophages. These data suggest that SelenoW is able to promote bacteria growth in macrophages by an unknown mechanism. Interestingly, integrated pathway analysis of



transcriptomic and metabolomic data of Ft-infected macrophages suggested a role of autophagy in bacterial growth. Interestingly, Ft is known to derive nutrients via autophagy when enclosed within *Francisella*-containing vacuoles. Furthermore, selenium deficiency has been implicated in inducing autophagy. Our preliminary studies have shown that macrophages derived from selenoprotein-deficient mice have elevated levels of autophagy markers suggesting selenoproteins regulate autophagy during infection. Together, we believe that multiple selenoproteins play a differential role during Ft infection and it is critical to dissect the specific role of individual selenoproteins. Our future work is directed towards establishing the specific role of selenoproteins by using unique specific selenoprotein-deficient mouse models. These studies will provide a broader understanding of pathogenies of tularemia and are likely to reveal novel targets for controlling infection and pathology.

43 **Same R0 but different epidemic dynamics? Revealing the role of correlation between host heterogeneity in transmission and susceptibility**

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Department of Biology, Penn State University

Some epidemics are fast and explosive while others are slow and meandering. While this difference could arise from differences in the basic reproductive number R0 (i.e., the average number of infections caused by one infectious individual in a fully susceptible population), here we explore whether correlations between host heterogeneities can also drive such patterns. It is well known that heterogeneity in transmission (i.e., different likelihoods of transmitting a pathogen) and heterogeneity in susceptibility (i.e., different likelihoods of being infected) separately affect disease dynamics, but correlations between them may have further implications. We explore the effects of these correlations on the epidemic size and the peak first with a systematic literature review and then by developing a model. We found disagreements and gaps in the published literature regarding the impact of correlations on disease dynamics. Therefore, we developed a stochastic individual-based SIR (Susceptible-Infected-Recovered) model that incorporates heterogeneity in both transmission and susceptibility and flexibly allows for positive or negative correlations between them. For a variety of heterogeneity and R0 values, we used this model to explore the effects of positive and negative correlations on disease dynamics. We found that positive correlations result in larger epidemics and larger, earlier peaks than dynamics with the same heterogeneity levels but no correlations between them. Conversely, negative correlations result in smaller epidemics, smaller peaks, and lower probabilities of outbreaks than when heterogeneities are not correlated. We also show that major epidemic outbreaks are possible even for R0<1 when heterogeneities are positively correlated. Our findings fill existing knowledge gaps regarding the impact of correlations between host heterogeneities. Ultimately, large, fast epidemics may be caused by positive correlations while small, slow epidemics may be caused by negative correlations, even when the models have identical parameters. This work provides novel insight to better predict disease dynamics and develop strategies for public health interventions targeted to generate negative correlations between susceptibility and transmissibility.

44 **Selenium-Dependent Regulation of Immune Checkpoint Control in Acute Myeloid Leukemia**
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Acute myeloid leukemia (AML) is a hematologic malignancy with a 27 % 5-year survival rate for people 20yrs and older with AML. Despite great progress with new therapies like PDL1/PD1 blockade therapy, immune evasion of leukemic cells persists leading to disease relapse. Programmed cell death ligand-1 (PD-L1, CD274), the co-inhibitory ligand interacting with its receptor PD1, contributes to immunosuppression despite being in an immunocompetent environment. Our studies demonstrate the Selenium (Se)-dependent modulation of PDL1 expression in LSCs and PD1 expression in CD8+ T-cells and T-cell exhaustion. Highlighting a novel adjunct dietary approach to the enhance the existing checkpoint blockade immunotherapy.

45 **The Role of Selenoprotein W in Stress Erythropoiesis**
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Stress erythropoiesis is a compensatory physiological response to anemic stress that rapidly develops abundant new erythrocytes. Cytoprotective protection against redox toxicity is essential for the maturation of erythroblasts, especially during stress erythropoiesis. Selenium is an essential trace element that plays an important role in the human immune system as an antioxidant through its incorporation into selenoproteins as the 21st amino acid, selenocysteine. Previous studies have shown that selenium deficiency or the lack of selenoprotein W (SelenoW) leads to a defect in the recovery of anemia by impacting stress erythropoiesis. Our studies confirmed that SelenoW assists the recovery from anemia through promoting erythroblast maturation during stress erythropoiesis. Interestingly, in SelenoW-/- mice, the proliferation of stress erythroid progenitors (SEPs) was promoted as a way to compensate for the defective erythroblast maturation, while the differentiation of SEPs was not affected. Further studies suggested that lack of SelenoW could affect the erythropoietic niche including central macrophage as well as the development and maturation of erythroblasts through Wnt and Yap signaling pathways. This study clarifies a novel mechanism for SelenoW in efficiently regulating stress erythropoiesis, where dietary selenium supplementation could serve as a potential adjuvant therapy for anemia.

46 **Effects of *Schistosoma mansoni* cercaria on the host immune responses**
Megan Nitchman, Pengyu Liu, Kaile Jump, Parisa Kalantari
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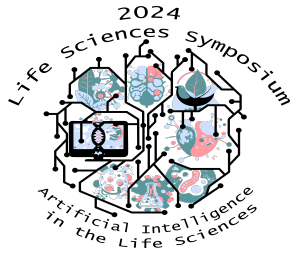
Schistosomiasis is a prevalent neglected tropical parasitic disease caused, in part, by the helminthic parasite *Schistosoma mansoni (S. mansoni)* affecting more than 240 million people globally. *Schistosoma mansoni* infections are known to cause both acute and chronic inflammatory stress in the mammalian host which impacts host ability to maintain homeostasis via immunoregulation. Some life cycle stages of the parasite have more characterized effects on



the host than others. In the context of established infection, eggs are known to have a highly inflammatory role resulting in the formation of hepatic granulomas, whereas adult worms have an immunosuppressive effect that contributes to parasitic survival in the host if left untreated. Currently, there is no consensus in the field as to whether cercariae have an inflammatory or immunosuppressive effect at the time of invasion. Cercariae are the only infectious stage to the mammalian host and thus have potential therapeutic implications for which disease prevention or early parasitic elimination could be targeted. It is difficult to work with cercaria due to their maturation volatility, meaning they easily transform into the next life cycle stage, schistosomula. Several groups tried to dissect the role cercariae and schistosomula have in disrupting host immunoregulation, but it remains unclear. While determining the best methods for maintaining viable cercariae, preliminary in-vitro data from our lab indicates that the presenting cercarial component dictates the host's cytokine response. Where insoluble cercarial material elicits a pro-inflammatory response and soluble excretory and secretory (E/S) products an anti-inflammatory cytokine release. We currently hypothesize that, *Schistosoma mansoni* cercaria invasion disrupts host immunoregulation via an acute cutaneous pro-inflammatory response which is rapidly dampened by E/S products to facilitate parasitic colonization in the host. Our ultimate goal is to elucidate the mechanisms by which cercaria disrupt host immunoregulation.

47 **Pharmacological activation of STING blocks schistosome egg-mediated pro-inflammatory cytokine production**

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Schistosomiasis is the second most prevalent parasitic disease around the world. The currently available treatments against this parasitic disease are through targeting worms. However, the infection with the helminth parasite *Schistosoma mansoni* causes morbidity and mortality via a pathogenic host CD4+ T cell-mediated immune response directed against parasite egg antigens. Therefore, a new treatment for regulating the immune response against parasitic eggs is vital in controlling the pathology of the disease. In the previous studies, we have demonstrated that low-pathology BL/6 mice lacking Stimulator of Interferon genes (STING) exhibited markedly enhanced hepatic granulomatous inflammation associated with significantly increased Th17 and diminished Th2 cytokine responses. Additionally, in contrast to BL/6 mice, CBA mice tend to have a severe form of liver granulomatous inflammation due to enhanced proinflammatory gene expression and a significant decrease in STING expression and type I Interferon (IFN) production. Since CBA bone marrow-derived dendritic cells have lower expression of STING, we hypothesized that STING agonists such as diABZI-3, would control the pro-inflammatory cytokine production and inflammation by activating STING in the high pathology model of schistosomiasis. Here, our results showed that the diABZI-3 induced robust IFNβ production, while IL-1β was impaired in egg-stimulated BMDCs. IL -17 expression was also hindered when co-culturing BMDCs with CD4+ naive T cells because of low IL-1β expression impaired by STING activation and IFNβ production. Furthermore, we found that the timing of diABZI-3 administration has a major impact on the outcome. The pretreatment of cells with STING agonist suppressed the IL-1β; while the

post-treatment of cells with STING agonist was no longer able to suppress this cytokine. Our in vitro and in vivo data suggest that the over-activation of STING by the diABZI-3 in BL/6 mice leads to a significant increase in IFNβ and IL-1β production and enhanced immunopathology. Our findings suggest that STING activation works as a double-edged sword: STING activation is important in suppressing IL-1β. but over-activating STING might lead to a pro-inflammatory environment. In conclusion, our study suggests that STING agonists may serve as a novel therapeutic strategy to restrain schistosome immunopathology in a high pathology model and the timing of STING agonist administration is essential in regulating the inflammation.

48 **The role of selenoproteins in neutrophils during Citrobacter rodentium induced colitis.**

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Inflammatory bowel disease (IBD) is characterized by chronic uncontrolled neutrophil (PMN)-associated inflammation in the gut. PMNs are innate immune cells that generate reactive oxygen species (ROS) upon activation, which modulates multiple pro-inflammatory and pro-resolving functions of PMNs. Crucial in fighting against invading pathogens, ROS can also induce host tissue damage when dysregulated. Selenium is an antioxidant that has been found to possess protective roles against IBD by regulating the functions of multiple immune cells in the form of selenoproteins, where selenium is translationally incorporated in the form of the 21st amino acid selenocysteine. To investigate the role of selenoproteins involved in the function of PMNs, we use a PMN-specific selenocysteine tRNA (TrspN) knockout mouse infected with murine pathogenic *Citrobacter rodentium* (*C. rodentium*) to induce colitis. Surprisingly, we observed an early clearance of the *C. rodetium* in the TrspN mice with increased tissue damage and PMN antimicrobial enzyme myeloperoxidase (MPO) expression in the colon. *Ex vivo* stimulation of TrspN PMNs showed upregulation of multiple proinflammatory mediators including Nos2, Il1b, and Tnfα, as well as increased extracellular ROS production and upregulation of antioxidant enzymes Sod2 and Gls2. Altogether, these results suggest that selenoprotein deficiency in PMNs resulted in increased ROS production upon inflammation, augmenting inflammatory response of PMNs thus promoting bacterial clearance, while compromising the resolution of inflammation, which leads to collateral tissue damage. Our findings uncover a protective role of PMN selenoproteome during the pathogenesis of *C. rodentium*-induced colitis providing potential therapeutic targets for IBD.

Microbiology and Virology

49 **Antimalarial Drug interactions with the Human Gut Microbiome**

Benjamin Anderson, Min Soo Kim, Jordan Bisanz
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Many common oral drugs elicit antibacterial effects and drug-microbe interactions in the gastrointestinal tract may limit their bioavailability. These interactions can contribute to

interindividual variation in drug response due to the highly individualized nature of microbiome compositions. Limiting bioavailability is worrying as it may result in sub-therapeutic serum concentrations of the drugs leading to treatment failure, or, in the case of infectious disease treatment, the development of drug resistant microbes. We have focused on antimalarial drugs as these compounds have high luminal concentrations, poor bioavailability, high rates of side effects, and drug-resistant malaria-causing *Plasmodium sp*. are on the rise posing a major public health threat. We hypothesize that bidirectional drug-microbe interactions between antimalarials and gut microbes shape microbiome composition and modulate the oral bioavailability of these compounds. Through sequencing and ex vivo experiments using human fecal material, we determined that there are extensive off-target antibacterial effects of common antimalarial drug families on gut microbial communities which could be replicated in humanized gnotobiotic mice. Notably, these antibacterial effects are donor-specific. To map these interactions, we performed high-throughput screening against a diverse collection of human microbes revealing both clade and strain-specific susceptibility patterns. Through pharmacokinetic analysis performed in gnotobiotic animals, we found that microbiome composition alters drug bioavailability. Taken together, these results show a bidirectional relationship between antimalarial drugs and the human gut microbiome. The ultimate goal of this project is to leverage understanding of drug-microbe interactions for personalized medicine and microbiota-targeted interventions to support the use of drugs we already have.

50 **Understanding and Manipulating Dietary Metal Bioavailability through the Gut Microbiome**

Daniela Betancurt-Anzola, Dean Miller, Jordan E. Bisanz
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Gut microbes shape the bioavailability of nutrients and xenobiotics consumed through the diet. Metals are ubiquitous in the diet and play important roles in host and microbial physiology; however, some metals like mercury are toxic to both. Microbial metal resistance often occurs through biotransformation reducing toxicity for both host and microbe. This is true of organomercurial lyase (MerB), an enzyme which demethylates highly bioavailable methylmercury (MeHg) to poorly-bioavailable forms. To better understand how the gut microbiome could be manipulated to modulate metal bioavailability, we analyzed the metal resistome of 398 lab isolates. While metal-resistance determinants are widespread, the genes for mercury biotransformation are rare and sporadically distributed with distant MerB homologs found in *Clostridia* isolate genomes. Based on these observations, we experimentally determined the important species and pathways involved in mercury biotransformation by exposing fecal samples from human donors to MeHg *ex vivo* and studying community growth and composition. We observed interindividual variation in susceptibility to MeHg and changes in community composition. Finally, to supplement the rarity of MerB in the gut microbiome, we have engineered strains of *Lacticaseibacillus* through chromosomal insertion of a constitutively expressed codon-optimized MerB derived from a highly MeHg-resistant *Bacillus megaterium* strain. We have confirmed the function of this construct hypothesizing that MerB results in intracellular accumulation of inorganic mercury which may then be carried out of the body by the

non-colonizing engineered strain. Our work is seeking to uncover how microbial biotransformation of metals affects their oral bioavailability and offers promise for microbiome-targeted interventions to improve human nutrition and health.

51 **Oxygen-sensing signaling proteins control metal homeostasis, cellular metabolism, and stress tolerance in two Gram-negative organisms**

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Bacteria utilize globin coupled sensor (GCS) proteins to sense and respond to oxygen and other diatomic gaseous ligands. A subgroup of GCS proteins contains a diguanylate cyclase (DGC) output domain which is linked to the sensory domain through a variable middle domain. DGC proteins synthesize the second messenger cyclic-di-GMP (c-di-GMP). C-di-GMP is known to influence motility, biofilm formation, and virulence, among other functions, in various bacteria. This work seeks to use DGC-containing GCS proteins as a model to further understand c-di-GMP signaling in Gram-negative bacteria. These proteins have been studied in various organisms, such as *Pectobacterium carotovorum* subsp. carotovorum (Pcc), an important plant pathogen. Pcc contains one GCS protein, the DGC PccDgcO, which synthesizes c-di-GMP upon oxygen binding. PccDgcO was previously shown to positively regulate flagellar motility, biofilm formation, and virulence factor excretion under aerobic conditions. Another DGC containing GCS protein, a homolog to PccDgcO, EcDosC, is found in the Gram-negative model organism *Escherichia coli* MG1655. EcDosC forms a complex *in vivo* with the gas sensing c-di-GMP phosphodiesterase (PDE), EcDosP. The EcDosCP complex is a unique c-di-GMP metabolic complex, in which both the DGC and PDE are active, and respond to the same signals, with different affinities. DosC and DosP have mainly been studied separately, together, it has been shown that they interact with RNase E and regulate RNA degradation *in vitro*. This work seeks to understand regulation of function by PccDgcO and the EcDosCP complex, using methods such as, RNA sequencing and differential expression analysis, ICP-MS, physiological assays, proteomics, and computational tools. Using these methods and knockout strains of the respective bacterial species, this work investigates the roles these proteins play in their respective organisms, to understand similarities and differences in the functions controlled in each species, and to use these proteins as models to understand localized vs. global c-di-GMP signaling in two distantly related organisms. Results show that both DGC proteins affect metal tolerance in their respective organisms, the first report of a DGC-containing GCS protein affecting metal homeostasis. Additionally, in *E. coli* the *ΔdosCP* strain shows a decreased tolerance to H2O2 under high Mn stress, highlighting the interaction of O2-sensing, c-di-GMP, and metal homeostasis. WT and *ΔdgcO* Pcc show differences in adaptation to changes in oxygen levels, showing a link between oxygen sensing, c-di-GMP signaling, and cellular metabolism. Additionally, this work includes the first use of the TurboID proximity labeling system in *E. coli*, revealing the protein-protein interactions of the DosCP complex, furthering the understanding of localized c-di-GMP signaling, with results validating *in vivo* DosCP's interaction with RNase E. These results, among others, reveal that GCS proteins with DGC output domains control a broad range of phenotypes relating to stress tolerance, biofilm

formation, and central metabolism, and highlight the broad role c-di-GMP plays in bacteria.

52 **The role of vitamin D in host resistance to H1N1 influenza infection**

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Department of Veterinary and Biomedical Sciences, Penn State University

Vitamin D, a fat-soluble nutrient, is vital for maintaining health by regulating the immune response, yet nearly half of the global population lacks adequate vitamin D intake through sunlight exposure, diet, and supplements, resulting in approximately one billion individuals suffering from deficiency in this essential nutrient. The precursor of vitamin D undergoes sequential metabolic transformations to generate its active form, 1,25-dihydroxyvitamin D (1,25(OH)2D), which then binds to the vitamin D receptor (VDR) to regulate gene expression in immune cells. Given the global health concern of influenza outbreaks and the prevalence of vitamin D deficiency in certain populations, this study investigates the role of vitamin D in host resistance to influenza virus infection, utilizing VDR knockout (KO) mice. In wild-type (WT) mice, H1N1 infection caused typical influenza symptoms but was non-lethal. Conversely, VDR knockout (KO) mice developed a severe infection with reduced survival (78%). VDR KO mice that survived to day 9 post-infection still expressed high levels of the influenza M gene in the lungs, whereas WT mice had cleared the virus. The lung tissue of VDR-KO mice showed more severe damage, with more alveolar congestion and hemorrhage. Additionally, VDR KO mice had alterations in lung immune cell populations, specifically an increase in macrophages, CD4 and CD8 T cells, but fewer Natural Killer (NK) cells than WT at day 9 post-infection. Together, the data suggest that VDR KO mice take longer to clear an influenza infection and have fewer NK cells but more inflammation than WT mice. Understanding the role of the VDR in controlling the lung immune response is crucial for advancing our understanding of the complex interplay between vitamin D and influenza. This study shows vitamin D status regulates immune response and susceptibility to viral infections, suggesting that vitamin D supplementation could serve as a low-cost, safe alternative to improve host resistance to respiratory viruses.

53 **Decoding a novel pathway for bile acid metabolism by the gut microbiome**

Min Soo Kim, Garrick T. Zhang, Jordan E. Bisanz
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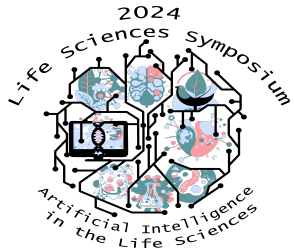
The complex interplay between the gut microbiome and the host is mediated by the constant exchange of macromolecules that are generated and modified by both microbes and mammalian cells, among which bile acids (BAs) hold significant importance. Gut microbes convert host-derived primary bile acids into secondary bile acids, such as 7α-dehydroxylated deoxycholic acid (DCA) and lithocholic acid (LCA), which are implicated in various diseases and pathogen infections. While the 7α-dehydroxylation of bile acids is attributed to bacteria encoding the bile acid-inducible (bai) operon, our preliminary findings suggest an alternative mechanism in a microbial community devoid of the bai operon. Our preliminary results indicate that a small microbial cohort of 37 bacterial strains is capable of producing 7α-dehydroxylated products in gnotobiotic mice and in culture, despite the absence of the bai operon. We hypothesize that a



subset of this community orchestrates the 7α-dehydroxylation reaction through an uncharacterized pathway, possibly involving polymicrobial cross-feeding of intermediates. Through combinatorial testing, quantitative LC/MS methods, and comparative genomics approaches, we aim to elucidate the chemical substrates and the microbial and genetic determinants necessary to complete this novel pathway. A deeper mechanistic understanding of this novel bile acid metabolism by the microbial community could significantly contribute to our knowledge of the origin of the abundant bioactive and physiologically relevant metabolites in the gut. Moreover, it could pave the way for the development of targeted therapies for gastrointestinal disorders and innovative strategies for pathogen management.

54 **Paramyxovirus-Like Particles for Therapeutic Delivery of Proteins to Cells**

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Virus-like particles (VLPs) have the potential to safely deliver functional proteins to cells for therapeutic purposes. However, foreign proteins do not naturally package into VLPs. We previously discovered that paramyxovirus NP proteins contain short packaging sequences near their C-terminal ends that can be fused with foreign proteins, allowing the foreign proteins to participate in viral assembly and package efficiently into VLPs. These VLPs in turn are naturally capable of delivering the cargo to the target cell interiors. Using this approach, we have generated paramyxovirus VLPs loaded with different cargos, including Cre recombinase. These VLPs can deliver biologically active cargo to target cell cytoplasm and nuclei. We tested multiple paramyxoviruses as VLP production platforms, including parainfluenza virus 5 (PIV5) and Nipah virus (NiV), and successfully achieved efficient cargo packaging and delivery in each case. We also tested a series of altered PIV5 M mutant proteins that have enhanced virus assembly capacity. These resulted in significantly improved VLP production and a corresponding increase in the delivery of functional Cre into target cell nuclei. Together, these findings lay the groundwork for the use of paramyxovirus-like particles as safe and effective tools for delivering therapeutic proteins to cells and tissues.

55 **Identifying the Lipoprotein N-acetylation Pathway in *Bacillus subtilis***

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Department of Biochemistry and Molecular Biology, Penn State University

Bacterial lipoproteins are ubiquitous and critical components of the membrane, accounting for approximately 3% of bacterial genomes with roles in nutrient transport, adhesion, germination, and sporulation, and much more. They are also important MAMPs (microbe-associated molecular patterns), as they are detected by Toll-like receptor 2 (TLR2), a membrane receptor expressed on eukaryotic cells that activates an immune response upon detection of foreign substances. Regardless of function, all lipoproteins share an N-acyl-S-(mono/di)-acyl-glyceryl-cysteine anchor which embeds them in the membrane. The N-terminal cysteine residue can be variably acylated, which can increase resistance to copper, a common antimicrobial agent, and decrease detection by TLR2, facilitating immune evasion. In some low GC-content Firmicutes such as *Bacillus subtilis*,

this cysteine can be acetylated, though the enzyme facilitating this post-translational modification was unknown. Using a TLR2-reporter assay, we identified three genes necessary for lipoprotein N-acetylation, which were verified with MALDI-TOF mass spectrometry: *pcrB, yvoF, and ypjA*. PcrB and YvoF were previously described to synthesize an archaeal-type lipid, acetylated heptaprenylglycerol, while YpjA is a protein of previously unknown function that shares weak homology to other acyltransferases. In our model, the acetylated heptaprenylglycerol produced by PcrB and YvoF acts as an acetyl shuttle across the lipid bilayer, allowing YpjA to transfer the high energy acetyl group to lipoproteins on the outer leaflet of the membrane. In addition to identifying residues critical for function of YpjA, we show preliminary data that the N-acetylation plays a role in germination of *Bacillus subtilis* spores, as knockouts of pcrB and yvoF display decreased nutrient-dependent germination efficiency.

Neuroscience

56 **Fos Contributes to Age-Related Memory Updating Impairments in Male Mice but Not Arc**

Alexandria McKenna, Chad Brunswick, Gretchen Pifer, Janine Kwapis
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Memory updating, or the ability to modify previously formed memories, significantly contributes to our accurate perception and navigation of the world around us. Age-related cognitive decline and various dementias impair memory updating, bringing about this stark reality for the older populations. Unfortunately, the molecular mechanisms behind age-related memory updating impairments remain unclear which, in turn, led us to design a memory updating experiment with a focus on genes – Arc and Fos – known to play critical roles in learning and memory. We hypothesized that expressions of Arc and Fos diminish in aging male mice, ultimately facilitating their memory updating impairments. Here, we used the Objects in Updated Locations (OUL) task with young adult (3-m.o.) and old (18-m.o.) C57BL/6J mice to model age-related memory updating impairments. After completing the OUL task, the mice were sacrificed and their brains were removed for dorsal hippocampus extractions. With the brain tissues, reverse-transcription quantitative polymerase chain reaction (RT-qPCR) analyses were completed to assess the expressions of Arc and Fos. We found that the expression of Arc remained consistent regardless of age, but expression of Fos significantly diminished in the aging male mice compared to the young adult male mice. These unexpected yet intriguing findings inspire future study to better understand why Fos contributes to age-related memory updating impairments but not Arc. Future experiments will manipulate the expressions of Arc and Fos in an attempt to rescue memory updating in aging male mice and attenuate memory updating in young adult male mice. These findings also prompted further investigation into identifying any other genes supporting age-related memory updating impairments; therefore, we are actively pursuing an RNA-sequencing experiment. Overall, this research has the exciting potential to improve treatments and therapies for those suffering from age-related cognitive decline and various dementias.

57 **Adolescent binge drinking alters prelimbic somatostatin neurons, but not adulthood alcohol consumption, in mice**

Avery Sicher, Keith Griffith, Matthew Springer, W. David Starnes, Nigel Dao, Grace Smith, Dakota Brockway, Laurel Seemiller, & Nicole A. Crowley
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Somatostatin (SST)-expressing neurons are a population of inhibitory neurons implicated in stress and neuropsychiatric disorders. We have shown that SST neuronal function in the prelimbic (PL) region of the prefrontal cortex is vulnerable to binge drinking in adult mice. However, the consequences of alcohol exposure during critical developmental windows, including adolescence, for SST neurons have not been characterized. Here, we investigated adolescent alcohol's effects on the intrinsic properties of PL SST neurons in mice. Based on our previous findings that PL SST neurons can mediate binge drinking in adult mice, we assessed the relationship between adolescent binge drinking and adulthood alcohol consumption. We used the Drinking-in-the-Dark (DID) paradigm to model binge drinking in male and female SST-Cre:Ai9 reporter mice from postnatal days (PND) 28 to 54. After adolescent DID, mice were assigned to patch-clamp electrophysiology or adulthood drinking experiments starting at PND 84. We found that SST neurons are hyperexcitable 30 days following adolescent DID. Despite these lasting electrophysiological changes, we did not see changes in binge drinking or preference for alcohol in adult mice which underwent adolescent binge drinking. Ongoing work seeks to elucidate how alcohol exposure interferes with typical SST development in adolescence. Understanding age-specific effects of alcohol exposure could lead to improved individualized and targeted interventions for alcohol use disorder.

58 **Continuous Actigraphy Tracking for Longitudinal Murine Sleep Architecture and Exercise Monitoring**

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Sleep patterns naturally evolve over the lifespan of an animal, but chronic sleep disruptions commonly develop as secondary factors underlying a variety of neurological diseases. Disruption of sleep – regardless of cause – has been identified as a risk factor for various neurological diseases and is potentially implicated in the mechanisms underlying disease progression. Prospective observational studies have shown that physical inactivity is one of the most common preventable risk factors for neurological disease. It is commonly accepted that exercise has positive benefits on sleep-wake regulation, but less is known about how other factors (i.e., sex, age, sleep quality, activity) mediate this complex system. Sleep and exercise interact through a series of complex, bidirectionally linked processes that affect multiple downstream physiologic pathways. We can probe the interactions between sleep and exercise in freely behaving animals using custom designed animal housing containing low-cost acquisition systems. This robust recording system is capable of continuously recording behavior and actigraphy from a large cohort of animals. From actigraphy, we can extract characterization of the animals' sleep which

we can use to track sleep architecture and exercise over the lifespan of the animals and to quantify aging associated changes. Activity and running are monitored at low resolution using video-based actigraphy tracking while higher resolution intracranial electroencephalogram (iEEG) can be added to experimental setups to allow for neuronal recordings. Using this system, we can quantify baseline measures of sleep architecture and general exercise, as well as how those measures shift over the course of aging.

59 **Investigating genes responsible for age-related memory updating impairments in the retrosplenial cortex**

Cyrus Marwaha, Alex McKenna, Chad Brunswick, Janine Kwapis
Department of Biology, Penn State University

Age-related memory impairments pose significant challenges to cognitive function, impacting daily life and contributing to age-related cognitive decline. This cognitive decline can often lead to detrimental effects including the inability to care for oneself and learn new things. Both of these outcomes come with a massive financial burden that can put an individual in economic hardship. Aging not only affects the formation of new memories, but it also interferes with the ability to modify previously formed memories. In our study, we focus on understanding the molecular mechanisms underlying these impairments, particularly in the retrosplenial cortex (RSC), a brain region with well-established roles in memory processing. To investigate molecular changes underlying these impairments, we used the Objects in Updated Locations (OUL) task to quantify memory updating in young and old mice. Older individuals commonly exhibit deficits in memory updating, which we have replicated in our OUL rodent task. We investigate how the expression of genes associated with memory updating differs between young and old mice, following memory updating. To unravel the genetic basis of these impairments, we conducted experiments involving memory updating tasks in mice, followed by molecular analyses of gene expression in the RSC. Specifically, we examined gene expression one hour after mice experienced memory updating, re-exposure to previously learned information, or were left in their home cages (control animals). Our ongoing investigation focuses on identifying genes that are upregulated in response to memory updating in young mice but not old mice. Prominent candidate genes include Fos, Arc, Per1, BDNF, Zif268, among others. Using qPCR, we aim to explore the differential expression patterns of these genes in the RSC across age groups and experimental conditions. Understanding the molecular signature associated with successful memory updating in young mice, contrasted with the impairments observed in old mice, holds profound implications for developing interventions to improve age-related cognitive decline. Future directions include exploring therapeutic strategies aimed at modulating the expression levels of candidate genes to potentially rescue memory updating deficits in aging individuals. This research underscores the importance of unraveling the intricate interplay between molecular processes and cognitive function, offering promising avenues for targeted interventions to preserve cognitive function across the lifespan.

60 **Repairing Age-Related Memory Updating Decline by Increasing Excitability of Neurons**

Derek Baldwin, Chad Brunswick, Janine Kwapis

Department of Biology, Penn State University

Previously formed memories can be updated to reflect new or changed information. Impairments in one's ability to update memories are common in many age-related diseases such as Alzheimer's and related dementias. Using the memory updating paradigm Objects in Updated Locations (OUL), we have observed that aging mice suffer impairments in their ability to update memories. We seek to characterize the mechanisms of age-related impairments in memory updating to better understand the biological process of memory updating. When memories are created, certain highly excitable neurons are selected to encode the information. Through processes such as long-term potentiation, these neurons become functionally linked in a group known as a neuronal ensemble. Ensembles are believed to be the physical counterpart to memory. During coallocation, ensembles from distinct memories can overlap one another, allowing the memories to be recalled simultaneously. We hypothesize that a lack of coallocation between ensembles belonging to the original memory and those belonging to update memories may be involved in many age-related memory updating impairments. We aim to restore memory updating effectiveness by increasing coallocation between original memory and update memory ensembles. By increasing the excitability of neurons belonging to a previously formed ensemble, newly forming ensembles can be directed to overlap with the original ensemble. Here we show two methods of increasing neuron excitability during learning which should allow us to restore updating ability by increasing ensemble coallocation. After neurons have been recruited into a neuronal ensemble, they retain a degree of residual excitability for a short time. In the first method we present a memory update shortly after training to leverage this residual excitability such that neurons belonging to the original memory will be preferentially selected to encode the update memory as well. This should increase coallocation between the original memory and the update and will hopefully improve memory updating. The second method utilizes the excitatory designer receptor (DREADD) hM3Dq to increase excitability of a neuronal ensemble during the formation of the original and update memories. This should bias that specific neuronal ensemble into being incorporated into both memories. We have successfully restored memory updating in old mice using these methods. The range of diseases capable of being addressed by these treatments would be much larger if the mechanism targeted by this treatment was similarly affected in old and young mice. To test this, we are repeating these experiments in young mice while using a subthreshold update. Subthreshold updates are shorter and therefore harder to learn, providing a target to rescue through treatment. Currently, around 50 million people across the world struggle daily with dementias such as Alzheimer's. Successfully restoring memory updating in aging mice by increasing excitability of neurons is a vital step in understanding the mechanisms dysregulated by these diseases. These results and future work will serve to further explain how updating mechanisms may act as a target for treatments of these diseases.

61 **Somatostatin Agonist Administration to the Prelimbic Cortex Modulates Drinking-in-the-Dark Behavior in Adult Mice**

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Department of Biology, Penn State University

Excessive alcohol consumption poses a serious risk to both individuals and society. Binge drinking, the most common form of excessive alcohol consumption, accounts for most of the negative consequences attributed to alcohol. The prefrontal cortex is a known nexus for decision making and a key region in our understanding of substance use in the brain. It has been shown previously that neuronal activity in the prelimbic subregion of the prefrontal cortex (PLC) is altered following alcohol consumption across various behavioral paradigms. Of particular importance is the shift in excitability of somatostatin (SST) neurons, a subset of inhibitory neurons located in the PLC. SST is implicated in a range of neuropsychiatric disorders and plays an important role in maintenance of the excitatory and inhibitory balance in the brain. Here, I utilize bilateral cannula infusion of SST agonist, Octreotide, into the prelimbic cortex throughout drinking-in-the-dark (DID) to modulate binge alcohol consumption in adult mice. Overall, infusions of low concentrations of Octreotide appear to increase alcohol consumption relative to control infusion while high concentrations show varied responses in modulating consumption.

62 **Two-photon imaging of noradrenergic neural control of cortical arterial dynamics in non-anesthetized mice**

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Sleep is characterized by global vasodilation of cerebral arteries with periodic pulsations that are thought to increase cerebrospinal fluid flow and glymphatic waste clearance. Dysfunction in this process contributes to the pathophysiology of many neurodegenerative conditions. Norepinephrine (NE) release from cortical axonal projections originating from the locus coeruleus (LC) precedes vasoconstriction and arousal from sleep. However, it is unknown whether local NE release from LC projections is necessary and sufficient to drive the vasoconstriction seen when awakening from sleep. This study used two photon imaging to observe cortical NE release alongside arterial diameter in the somatosensory cortex of head-fixed, non-anesthetized C57BL/6J mice as they sleep. We expressed a G protein-coupled Receptor-Associated Biosensor (GRABNE-2m), in the mouse somatosensory cortex and imaged through a thinned-skull window to visualize local noradrenergic tone around single penetrating arterioles. Vascular diameter was measured by transducing hepatocytes to secrete mScarlet-Albumin into the blood. Non-rapid eye movement (NREM) and rapid eye movement (REM) sleep states were monitored using electrocorticography, electromyography, pupillometry, and motion sensors. Preliminary results show that norepinephrine around penetrating arterioles was highest during wakefulness, decreased during NREM, and was lowest during REM sleep. Immediately prior to arousal from sleep, local norepinephrine levels rapidly increased back to waking baseline levels, followed by vasoconstriction. Future directions include optogenetic stimulation of LC neurons to determine if this is sufficient to reproduce the vascular dynamics seen in natural arousal from sleep.

Physiology and Nutrition

63 **Determining the role of RRN3 in Skeletal Muscle: A Necessary Mediator for Hypertrophy**
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Ribosome production is critical for skeletal muscle growth, with ribosomal (r)RNA transcription playing a key role in facilitating muscle protein synthesis. RNA polymerase I (Pol I) recruitment to the rDNA gene repeats is promoted by interactions with RRN3 which forms the Pol I pre-initiation complex. While RRN3-mediated Pol I recruitment is crucial for rDNA transcription, its significance for muscle growth remains unexplored. Therefore, to uncover RRN3's role in skeletal muscle, we conditionally inactivated it in myofibers using a transgenic mouse model that was generated by crossbreeding RRN3 floxed (RRN3f/f) mice with a tamoxifen inducible mutated estrogen receptor-flanked Cre recombinase under the control of the human α-skeletal actin gene promoter (RRN3f/f x HSA-MCM). At 8-weeks of age, RRN3f/f and RRN3f/f x HSA-MCM (RRN3Δ/Δ) littermates were treated with 2mg of tamoxifen in corn oil for five consecutive days and subsequently placed on a tamoxifen-based chow (500mg/kg) until their collection at 24 weeks of age. In a separate experiment, mechanical overloads (OL) were performed by removal of the lower third of the soleus and gastrocnemius muscles to induce muscle hypertrophy of the plantaris muscles and a minimally invasive sham procedure served as a control. The OL and sham surgeries were performed on RRN3f/f and RRN3Δ/Δ mice at 12 weeks of age and were collected after either 3- or 14-days. Total RNA was extracted from gastrocnemius and plantaris skeletal muscles, quantified spectrophotometrically, and analyzed via qPCR. Plantaris muscles were analyzed for muscle fiber cross-sectional area (CSA) using Hematoxylin and Eosin staining and quantified on ImageJ. Chromatin Immunoprecipitation (ChIP) was performed on 5ug of plantaris chromatin to assess Pol I binding to the rDNA repeat. Inactivation of muscle fiber RRN3 led to a significant decline in overall body weight when compared to RRN3f/f mice. This was accompanied by lower plantaris and gastrocnemius muscle weights, and a lower mean plantaris muscle fiber CSA. Additionally, we found decreased total rRNA levels, reduced 45S pre-rRNA transcription, and a lower expression of the 18S, 5.8, and 28S rRNA's. Since the inactivation of RRN3 did not entirely abrogate rDNA transcription at rest, we then examined the role of RRN3 in muscle hypertrophy. Following 14-days of OL, RRN3Δ/Δ mice showed a diminished hypertrophic response, exhibiting 32.20% less muscle growth and a 35.75% lower mean muscle fiber CSA. Translational capacity (rRNA) was significantly impaired after 3- and 14-days of OL in addition to significantly less ETS and ITS 45S pre-rRNA transcription, and 18S, 5.8S, and 28S rRNA expression. Finally, binding of Pol I at various regions of the rDNA repeat was significantly decreased at the rDNA promoter, 5'ETS, 18S, ITS1, 5.8S, 28S, and 3'ETS regions of OL RRN3 null mice when compared to the RRN3f/f group. These findings demonstrate RRN3's crucial role in promoting enhanced rDNA transcription during skeletal muscle hypertrophy. However, its impact on myofibers at rest appears to result in only minor impairments.

64 **High-fat diet- and LPS-induced chronic inflammation elicits changes in hydroxyeicosatetraenoic acids (HETEs) in the cerebrospinal fluid and plasma of Sprague-Dawley rats**
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Chronic neuroinflammation is a well-documented and abundant pathology of neurodegenerative disorders. Metabolites of arachidonic acid, an omega-6 polyunsaturated fatty acid (PUFA), can be released from cells of the central nervous system. These metabolites can exacerbate pro-inflammatory microglial phenotypes and thereby increase pro-inflammatory cytokine production, further aggravating a state of chronic neuroinflammation. For this study, we wanted to evaluate the changes in PUFA-derived oxylipins in Sprague-Dawley rats responding to diet-induced or endotoxin-induced inflammatory challenges. This allowed us to compare each type of inflammation and the oxylipins it produces, as well as sex differences in oxylipin production. The rats were randomized into four groups, each group having 12 males and 12 females (n = 24 per group). The HFD group was fed a high-fat diet for six weeks consisting of 20% carbohydrates, 20% protein, and 60% fat, containing high levels of saturated fatty acids and trans-fats and low levels of polyunsaturated fatty acids. This was compared to a reference chow diet (CHOW). Chronic inflammation was modeled by administering saline injections (VEH) or lipopolysaccharide injections (LPS) every other day for three weeks, and these rats were given the same chow diet as the CHOW group. Targeted lipidomic analyses were conducted in the cerebrospinal fluid and plasma of rats, and oxylipin concentrations were analyzed via LC-MS/MS. 5-HETE, 12-HETE, and 15-HETE, which are arachidonic acid metabolites of 5-lipoxygenase (LOX), 12-LOX, and 15-LOX, respectively, were measured. HFD induced a 5-fold increase ($p < 0.0001$) in 5-HETE in the plasma, but not in the cerebrospinal fluid ($p > 0.80$). Rats displayed a 5-fold difference ($p < 0.0001$) in 5-HETE production in response to LPS injection in the cerebrospinal fluid and plasma; moreover, females exhibited an additional 2-fold greater difference ($p = 0.005$) in 5-HETE production in the cerebrospinal fluid compared to male-LPS challenged rats. Interestingly, HFD rats had nearly the same amount of 5-HETE in plasma as LPS rats. Compared to 5-HETE, 12-HETE production in the cerebrospinal fluid of both HFD- and LPS-treated rats was 5-fold greater than control conditions, and HFD rats had nearly the same amount of 12-HETE in plasma as LPS rats. In contrast, plasma 12-HETE exhibited a 5-fold greater response ($p < 0.0001$) in LPS-challenged rats compared to HFD, with females exhibiting an additional 5-fold greater difference ($p < 0.0001$). Surprisingly, there was also a 5-fold difference ($p < 0.0001$) in 12-HETE in females compared to males in the vehicle group, which suggests a fascinating female-specific response to the act of injection in plasma. Lastly, there were no biologically relevant differences in 15-HETE production following inflammatory challenge. This data shows that both 5-HETE and 12-HETE respond to endotoxin-induced inflammatory challenge in both the plasma and cerebrospinal fluid; alternatively, diet-induced inflammatory challenge only provokes a 12-HETE response in both the cerebrospinal fluid and plasma, whereas a diet-induced response in 5-HETE is only elicited in the plasma. The data also shows that females exhibit greater differences in oxylipin production specifically when receiving an endotoxin-induced inflammatory challenge.

65 **NLRP3 Inflammasome Inhibition Reduces Ischemia/Reperfusion Injury in Female but not Male Rats**

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Background: Myocardial infarction (MI), or “heart attack”, remains a leading cause of death worldwide. MI is caused by prolonged coronary artery ischemia (I; blockage of blood flow) leading to cell death. While reperfusion (R) is therapeutic, continued activation of cell death pathways through distinct and overlapping mechanisms exacerbates damage beyond that due to I. The NLR family pyrin domain containing 3 (NLRP3) inflammasome is activated during I/R, leading to pyroptosis. However, NLRP3 inhibition beyond 2 hr R fails to reduce MI size. Therefore, we sought to determine whether limiting NLRP3 priming prior to I is sufficient to reduce I/R injury and MI size. Purpose: To investigate the protective efficacy of NLRP3 gene knockout (KO) and loss of NLRP3 priming on MI size in male vs female rats. Methods: NLRP3 KO DAHL/SS-Nlrp3em2Mcwi rats were generated via CRISPR-Cas9 (14 base pair deletion in exon 1 of NLRP3 gene) in Dahl salt sensitive rats. Wildtype (WT) and NLRP3 KO rats received Teklad 7034 low salt diet (0.12% NaCl) ad libitum to delay the development of hypertension. *In-vivo* coronary artery ligation (CAL; 33 min I) was used to induce MI in male (n=9) and female (n=12) WT (n=10) and NLRP3 KO (n=11) rats aged 90-135 days, with sham surgery as a non-ischemic control. Cardiac puncture was used to collect blood following 3 hr R to assess IL-18 levels and complete blood count (CBC). The left ventricle was harvested to determine area at risk (AAR) and infarct size using Evans Blue dye perfusion and 2,3,5-Triphenyltetrazolium chloride (TTC) staining, respectively. AAR and infarct size quantification was conducted using ImageJ, with data analyzed via 2-way ANOVA (Minitab). Data were presented with mean ± SEM. Results: NLRP3 KO had increased left ventricle weight (LVW)/right tibia length (RTL) (male: 244.32 ± 6.29 mg/cm; female: 202.02 ± 5.06 mg/cm) vs. WT (male: 233.26 ± 4.34 mg/cm; female: 191.11 ± 3.69 mg/cm; $p < 0.05$). Baseline plasma IL-18 levels were elevated in NLRP3 KO (male: 21.54 ± 2.00 pg/mL; female: 19.36 ± 1.80 pg/mL) vs. WT (male: 9.06 ± 2.01 pg/mL; female: 5.13 ± 1.55 pg/mL; $p < 0.05$). Infarct size was reduced in NLRP3 KO vs WT females (KO vs. WT: 20.06% vs. 24.53%), but not males (KO vs. WT: 34.27% vs. 28.06%). Blood neutrophils were increased in all groups after MI (Baseline vs. I/R: WT female: 23.70 ± 2.75% vs. 40.84 ± 6.86%; NLRP3 KO female: 24.81 ± 1.74% vs. 48.37 ± 8.43%; WT male: 27.10 ± 1.82% vs. 66.07 ± 0.03%; NLRP3 KO male: 24.81 ± 1.74% vs. 66.19 ± 1.20%). Conclusion: The absence of NLPR3 may be cardioprotective in female but not male rats. Elevated IL-18 levels at baseline in NLRP3 KO require further investigation, but suggest dysregulated Interferon-gamma expression and/or alternative inflammasome activation, such as AIM2. Understanding divergent roles of NLRP3 inflammasome activation may inform sex-specific therapeutic interventions for the treatment of cardiac inflammation and MI. Our data also suggest a possible direct role for NLRP3 in the regulation of cardiac growth.

66 **Phytochemical Analysis of Pennsylvania “Ramps”, Allium tricoccum and A. burdickii.**

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“Ramps” is the common name for two species of wild Allium (*A. tricoccum* and *A. burdickii*) which are commonly harvested from the forests of Appalachia and northeastern and north central

United States. For this study, I extracted both species of ramps and used high pressure liquid chromatography mass spectrometry (HPLC-MS/MS) in the Huck Metabolomics Core to compare their phytochemistry. The water-soluble vitamin content of both species was also quantified using HPLC with diode array detector and standard curves. Overall, both species were found to be high in vitamin C and to contain many B vitamins. Metabolomic analyses using MS Dial, GNPS.ucsd.edu, and Metaboanalyst.ca, revealed the differences between the species' sulfur profiles which could explain flavor differences. Additional classes of compounds tentatively identified and compared in the ramps include steroidal saponins, polyphenols (including anthocyanins), and sphingolipid ceramides. This helps inform the taxonomy and delineation of the two species and informs consumers about nutritional and bioactive compounds in ramps.

67 **Sex differences in high-fat diet induced dysbiosis**

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Chronic consumption of high-fat diet (HFD) is a potent modulator of gut microbiota. While this interaction is well established in male animal models, less is known about the impact of high-fat obesogenic diet on female microbiota. Considering that some sex differences exist in the microbiota of lean males and females and that considerable sex differences exist in the pathophysiology of obesity in males and females, it is likely that their gut microbiota response will also differ. Here we aim to investigate sex differences in the high-fat diet induced gut dysbiosis. Adult male and female rats were fed HFD (60%) or standard chow diet for six weeks which led to significantly increased food intake and body weight, as well as inguinal and gonadal white adipose weights between the diet groups at 6 weeks from diet initiation. Fecal samples were also collected at 6 weeks, to examine the differences in microbiota using 16S rRNA analysis. We found that alpha-diversity metrics, comparing evenness and richness of microbial species, were decreased in HFD-fed females compared to chow-fed females. However, in males, HFD produced a trend to increased evenness and only marginal changes in richness. Beta diversity, an initial indication of a shift in the microbial community composition between cohorts, was altered by HFD to a similar extent in both sexes, where HFD-fed males and females displayed a distinct separation from their chow-fed counter parts. Abundance of over 60 taxa was reduced by HFD in females, and another 40 were increased in females by the HFD. While the numbers of altered taxa were similar in males, the specific taxa affected were largely entirely sex divergent. Further analysis of the cytokines and metabolites in the plasma and the brain was performed to determine the primary microbial drivers of each parameter per sex. Our data indicate drastic differences in the specific effects of HFD on the gut microbial community, with links to systemic and central metabolism and inflammation.

68 **Harvesting Health: Identifying Aryl Hydrocarbon Receptor Modulators in Edible Fungi to Illuminate the Path to Gastrointestinal Wellness**

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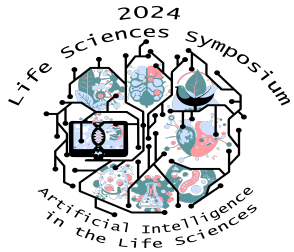

Significance: The aryl hydrocarbon receptor (AHR) is a nuclear receptor that has been shown to have numerous biological functions including cell cycle regulation, liver development, circadian rhythm regulation, as well as gut homeostasis regulation. Importantly, AHR is abundant in the gastrointestinal tract, and diet-derived AHR ligands have potential to maintain homeostasis in the gut. Foods are significant sources of AHR modulating compounds, rich in phytochemicals and mycochemicals. However, among the myriad bioactive compounds from edible plants and fungi, identifying novel functional ligands which would significantly impact gastrointestinal health is a challenge. Purpose: Using a combination of untargeted metabolomics, molecular networking, molecular docking, and data mining, predict biologically functional compounds accurately and efficiently. Methodology: Mass Spectrometry (MS) data from *Agaricus bisporus* (white button mushroom) methanol extract was processed through Global Natural Products Social Molecular Networking to predict the novel compound. AHR activation was measured by luciferase-based reporter assay on HepG2 40/6 and Hepa 1.1 cells. CYP1A1 gene expression was confirmed via RT-qPCR. Compound isolated by HPLC and identified by LC-MS and 1H NMR. Unpaired t-test used to analyze *in vitro* experiments. Dose-response activity of *Ganoderma lucidum* (Reishi mushroom), *Pleurotus ostreatus* (oyster mushroom), and *Hypsizygus tessellatus* (beech mushroom) methanol extracts were also screened for AHR activity with luciferase-based reporter assay, and their metabolomic profiles were analyzed by UHPLC/LC-MS. Regression analysis was applied to the MS1 data to identify compounds correlated with greater receptor modulating capabilities, and MS2 was screened to match against compounds libraries for identification. Results: A novel AHR modulator in the white button mushroom (*Agaricus bisporus*) has been predicted, identified, and characterized, and by these methods. The metabolome of *Agaricus bisporus* was analyzed in combination with known AHR ligands to find structural associations between the compounds constituting the two groups. Molecular networking of these compounds revealed that a methylated analog to benzothiazole was indicated in *Agaricus bisporus*, which was subsequently isolated and identified as 2-amino-4-methyl-benzothiazole(2A4). Cell-based AHR transcriptional assays revealed that 2A4 possesses agonistic activity and upregulated CYP1A1 expression. Using the same set of protocols, more fungi have been studied for novel AHR modulators with nutritional and historical medicinal significance. Luciferase reporter assays on such fungi have revealed that *Ganoderma lucidum* (Reishi mushroom), *Pleurotus ostreatus* (Oyster mushroom), and *Hypsizygus tessellatus* (Beech mushroom) all have significant dose-responsive AHR modulating activity in hepatocytes and are prime candidates for sources of novel AHR modulating compounds using this method. PLS-R modeling of the metabolome of both oyster and beech mushrooms have revealed upwards of 20 unique compounds that may be responsible for receptor activation. Conclusions: Machine learning models such as linear regression have the power to simplify a complex set of multidimensional data and separate identify unique points of interest to tease out compounds in natural products research. *Ganoderma lucidum, Pleurotus ostreatus*, and *Hypsizygus tessellatus* all have the potential to guide dietary advice in maintaining gut health and systemic health through the multitude of biological functions AHR regulates.