Chemistry & Biochemistry Poster Fair Presenters Abstracts

Tuesday, April 29, 2025
1:00pm – 3:30pm
Bear Down Gym

THE UNIVERSITY OF ARIZONA
COLLEGE OF SCIENCE
COLLEGE OF MEDICINE TUCSON
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2025 CBC Senior Thesis/Capstone Presentations

Abdulqadir Ali – Biochemistry #22

Research Faculty Advisor: Leif Abrell, Environmental Science and Chemistry and Biochemistry

PFAS Contamination Analysis in Fish Tissue

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants increasingly detected in seafood, raising concerns about human dietary exposure. This study investigated two primary questions: whether preservation methods (freezing and canning) influence PFAS levels in sockeye salmon, and how reliable the extraction and analysis methods are for quantifying PFAS in fish tissue. Using EPA Method 1633, homogenized samples of fresh, frozen, and canned salmon were processed and analyzed through liquid extraction, solid-phase extraction and LC-MS/MS. The results showed that while several compounds were reliably detected, PFAS concentrations varied across sample types, with fresh fish not always exhibiting the lowest levels. Our findings suggest that PFAS contamination likely stems from environmental exposure rather than being introduced during preservation.

Janelle Amegatse – Chemistry #9

Research Faculty Advisor: Thomas Gianetti, Chemistry and Biochemistry

Tricationic Dimethoxyquinacridinium (DMQA+) for an Aqueous Redox Flow Battery

Novel, long-duration energy storage solutions are mandated by the intermittent nature of renewable energy sources, such as wind and solar. Herein, the usage of a tricationic, organic dimethoxyquinacridinium (DMQA) is explored as the redox active molecule for a symmetric redox flow battery due to its bipolar nature. The correlation between the supporting electrolyte's identity and concentration and this DMQA's electrochemical performance, such as the diffusion coefficient and open circuit voltage, was studied.

Gavin Arnold – Biochemistry #59

Research Faculty Advisor: Ying-Hui Chou, Psychology

Transcutaneous auricular vagus nerve stimulation as a primer for neural plasticity—Parameter review and study protocol for a sham-controlled, randomized trial

Transcutaneous auricular vagus nerve stimulation (taVNS) is a novel non-invasive brain stimulation tool that utilizes the auricular branch of the vagus nerve to modulate its activity within the body via the ear. While the exact mechanism of this stimulation's effects remains unknown, growing evidence supports the use of this device as a modulator of neural plasticity: a brain feature with paramount importance in memory formation and learning. taVNS thus represents a burgeoning frontier in rehabilitation efforts for individuals suffering from loss of memory and cognition. Due to the novelty of this device, an ongoing challenge in its adaptation for research use is the lack of established parameters relating to frequency, pulse width, duration, and intensity among others. The widespread variation between these parameters in studies engenders a lack of robust evidence to confirm the significance of taVNS' effects. Thus, considerable research must be completed to determine optimal parameters and establish them as the foundation for taVNS research. This work aims to further these efforts by reviewing the current literature surrounding the use of varying frequency parameters in taVNS protocols in human subjects research for cortical excitability: a marker of neural plasticity. Then, a clinical trial protocol is proposed to experimentally determine the effects of different frequency values of taVNS on neural plasticity via transcranial magnetic stimulation (TMS).

Benjamin Augustine – Chemistry #24

Research Faculty Advisor: Vicente Talanquer, Chemistry and Biochemistry

Mechanisms of benefit acquisition for students involved in chemistry based undergraduate research experiences

Current literature on Undergraduate Research Experiences (UREs) explore the positive outcomes of such experiences, which fall primarily into 2 categories: those which produce increases in tangible skills and knowledge, and those which produce increases to the internalized benefits of self-perception, mindset and self-efficacy. The mechanisms as to how students perceive these benefits is less well understood. This study postulates that students benefit from UREs through Bandura's social learning and self-efficacy theories. Research on undergraduate students (n=991) enrolled in biochemistry and chemistry at a four-year university found that participation in research benefited students' cumulative GPA by 0.24 percentage points on average. Qualitative interviews with students who participated in UREs found that students who participated in UREs perceived benefits in their research and presentation skills, and in their views on their own competence and confidence as chemists. These benefits were actualized due to social learning, through interactions with peers and mentors, and through self-efficacy, as they grew in mastery of the various lab procedures and operations.

Ryenne Belt – Biochemistry #46

Research Faculty Advisor: Julie Armin, Family and Community Medicine

Receipt of and Biochemical Screening Methods of Human Papillomavirus Testing and Cervical Cancer Screenings in Arizona Tribal Lands

This study examines the availability of cervical cancer screening and HPV testing for Native American women in Arizona, focusing on biochemical screening technologies. Native American women experience disproportionately high cervical cancer mortality rates due to limited access to screening. This research integrates quantitative data analysis on publicly available screening rates, qualitative insights on current legislation that aids in the financial barrier to care, and biochemical evaluations to assess the accessibility and effectiveness of current and emerging screening technologies. I will analyze public data on screening methods by geographic region to assist in the identification of current disparities and find potential areas for improvement. I will evaluate the potential applications of certain HPV tests- such as the Cobas HPV test and Hybrid Capture 2 (HC2) - in rural settings taking in current screening rates, the technology that exists in these health care centers, and policy frameworks.

Katelyn Boone – Biochemistry #61

Research Faculty Advisor: Stephen Cowen, Psychology

Dorsal hippocampal single-unit responses to time, distance of string pulled, and trial condition during a novel fine-motor string-pulling behavior

Spatial encoding and retrieval is associated with the hippocampus, which is believed to underlie episodic memory. Past research has shown that the hippocampus encodes several dimensions such as space, time, distance, and auditory frequency. However there is no research in the role of hippocampal single unit-activity during fine-motor paw movement and abstract distance during pulling behaviors. Animal models were trained to use an infinite pulley-driven string-pulling apparatus. Once trained properly rats were implanted with neuropixels in the CA1 and CA3 region of the hippocampus. Our results demonstrate that hippocampal CA1 and CA3 neurons respond to brief moments in time from the start of a string-pulling bout and segments of distance of string pulled. These results complement growing evidence conveying that the hippocampus encodes dimensions beyond spatial location, and that it can flexibly map onto segments of time and distance of string pulled.

John Casey – Biochemistry #30

Research Faculty Advisor: Thomas Tomasiak, Chemistry and Biochemistry

Identification of Novel Inhibitors Targeting the Valley Fever Protein Cps1 Using Molecular Modeling Techniques

Identification and characterization of novel inhibitors targeting CPS1: Implications in developing potential therapeutics against Coccidioides posadasii

The fungal pathogens C. immitis and C. posadasii are known to cause coccidioidomycosis, commonly referred to as Valley fever. Currently, this disease impacts a dozen states on the West Coast of the United States, and by the year 2095, the majority of the West Coast is projected to be considered endemic. While most infected patients experience mild respiratory distress that resolves spontaneously, there are some cases in which the spores disseminate from the lungs through the bloodstream. These cases are often long-lasting and, unfortunately, commonly have fatal outcomes. This combination of a rising number of cases and potentially fatal outcomes illustrates the need to identify new therapeutics aimed at the treatment of Valley fever. More specifically, our research focuses on a novel treatment that attempts to disrupt key proteins responsible for Coccidioides' virulence and key cellular activities.

Recently, a mutagenesis with the fungal maize pathogen C. heterostrophus introduced CPS1, a protein having putative roles in critical cellular functions, such as cell wall formation. In another recent study, a mutant strain of C. posadasii lacking the CPS1 gene showed promise when it failed to cause disease in both healthy and immunocompromised mouse models. This illustrated the protein's effects on the virulence of Valley fever and the ability of the disease to persist without CPS1. The following study presents our data on the identification and in silico characterization of potential inhibitors of CPS1 through computational approaches. These methods include a combination of molecular modeling, screening ligand databases, simulating docking sequences, and testing molecular dynamics. The best hits exhibiting favorable binding energies and inhibitory effects will hold immense potential in inhibiting the functionality of CPS1 while giving a basis for future in vitro and in vivo studies. Additionally, our research shows our results when attempting to express and extract the Cps1 protein. From this, we hope to further streamline our approach in extracting Cps1 using fungal cells and further test our Cps1 inhibitors.

Christian Chan – Biochemistry #63

Research Faculty Advisor: Jeong-Yeol Yoon, BioMedical Engineering

Paper-Microfluidic Platform with Cloud-Based Capillary Flow Analysis for Detection of HPV 16 Towards Oropharyngeal Cancer Screening

Human papillomavirus (HPV) is an oncogenic virus that can lead to the development of various types of cancers, including oropharyngeal cancer (OPC). However, there are currently no tests that allow for the rapid and accurate detection of HPV-positive OPC. This is problematic because HPV-positive OPC cases have been continuously increasing. In these sets of experiments, HPV 16 DNA was amplified through recombinase polymerase amplification (RPA) to run on paper microfluidic chips while a smartphone was used to record the flow of our samples. From these video recordings, capillary flow velocity analyses were performed using a custom Matlab script which differentiated HPV 16 samples from no target control (NTC) samples. In order to be able to identify the HPV 16 samples from the NTC samples, amine-functionalized microspheres of diameters 0.5 µm and 1.0 µm were preloaded on the paper microfluidic chips or SYBR Green dye was used. The initial slopes of the flow velocity profiles and the SYBR Green dye resulted in the greatest differences between NTC and HPV samples, as well as between varying DNA lengths.

Sean Chen – Biochemistry #1

Research Faculty Advisor: Michael Brown, Chemistry and Biochemistry

GPCR signal transduction is affected by water and lipids

Rhodopsin is a prototype for the largest family of G-protein-coupled receptors (GPCRs), which are highly important to cellular signal transduction. Using UV-visible spectroscopy, we investigated the binding to rhodopsin of peptide analogues of the G-protein, transducin. As previously demonstrated, rhodopsin activation is related to a large influx of bulk water molecules (~80) and is affected by lipid membrane composition. Our work has further shown the significance of soft matter (lipids and water) for signal transduction. By using hydrophilic polymers of varying molecular weight (polyethylene glycols), we demonstrated the effects of hydration on signal transduction. Large osmolytes dehydrate rhodopsin, shift the activation equilibrium back to inactive state, and decrease the binding affinity of G-protein peptides. Small osmolytes penetrate the transducin binding pocket of rhodopsin, forward shifting the equilibrium to the active state. Their lower conformational entropy promotes increased binding of peptide until reaching the saturation point. We also studied rhodopsin recombinant membranes to test the effect of the lipid environment as described by the flexible surface model. Phosphatidylcholine membranes with ≈ zero intrinsic curvature decrease the binding affinity between rhodopsin and peptide, while negative intrinsic curvature of native lipid membranes increases the binding. Combined effects of osmotic stress and lipids were also investigated. The POPC membranes universally decreased binding affinity whereas the effects of large or small osmolytes remained as described above. Membranes with negative intrinsic curvature universally increased binding affinity which was still affected by osmotic stress. Curvature forces had significant effects on the activation of rhodopsin and transducin binding but were outweighed by osmotic stress. We additionally observed that the binding affinity of the G-protein peptide was significantly decreased by POPC lipids and osmotic dehydration compared to the native environment.

Dorie Chen – Biochemistry #60

Research Faculty Advisor: William (Scott) Killgore, Psychology

"The Impact of Sleep-Wake Variability on Reaction Time in Psychomotor Vigilance Tasks: An Actigraphy-Based Study"

Cognitive decline as a result of sleep deprivation poses a significant risk to military personnel, who often endure extended periods of wakefulness in the field. This study explores the relationship between total sleep time and sleep-wake cycle variability versus response speed (RS), a key measure of cognitive performance. Using actigraphy to measure total sleep time (TST) and sleep variability, we hypothesized that individuals with more consistent sleep-wake cycles and longer sleep durations would exhibit higher reaction speeds on Psychomotor Vigilance Tasks (PVTs) compared to those with shorter sleep durations and greater sleep variability. A randomized double-blind study involving 150 participants (military and non-military personnel) was conducted, with data collection spanning 13 days. Actigraphy provided insights into participants' sleep patterns, while PVT tasks measured RS at six timepoints throughout the day (8am, 11am, 2pm, 5pm, 8pm, 11pm). Linear regression analyses were performed to examine the relationship between TST, sleep variability, and RS. However, the analysis did not yield statistically significant findings, suggesting that observed variations in the data were likely due to random fluctuations. Possible explanations for the lack of significance include averaging of data, sample size limitations, and methodological factors. This study underscores the need for further research with refined methodologies and larger sample sizes to explore potential trends in the relationship between sleep variability and cognitive performance.

Kay Do – Chemistry #19

Research Faculty Advisor: Elisa Tomat, Chemistry and Biochemistry

Synthesis and Characterization of Metal-Binding Biopyrrin Pigments

Tetrapyrrolic bile pigments have been a well-studied class of oligopyrroles since their initial isolation in the 19th century, playing important roles in biological processes such as the degradation of heme and chlorophyll. In the structure of these naturally occurring oligopyrroles (e.g., biliverdin, bilirubin), the pyrrole rings are linked by methylene or methine bridges at the alpha position, and the terminal pyrrole rings feature carbonyl groups. Among the tripyrrolic compounds, lower-order analogs, such as tripyrranes, tripyrrins, and tripyrrin-1,14,diones, have been an emerging class of redox-active ligands in coordination chemistry, biological ion acceptors or transporters in medicinal chemistry, and building blocks for expanded porphyrins in synthetic chemistry. The tripyrrindione, a tripyrrolic synthetic analog of the uroerythrin pigment, has been shown to coordinate with divalent metals, such as Pd(II), Zn(II), and Pt(II), as a dianionic radical. Synthetic modifications of the tripyrrindione scaffold are expected to modify the electrochemical profile and coordination chemistry of this ligand.

For my thesis, I sought to investigate the synthetic and coordination chemistry of the tripyrrindione scaffold. Previously reported methods for the synthesis of these biopyrrin precursors were optimized towards the formation of supramolecular assemblies. Hexaalkyl tripyrrin-1,4-dione was synthesized to coordinate with silver(I) acetate as a tridentate, monoanionic radical. Meso-aryl tripyrrindione featuring pentafluorophenyl substituents was additionally explored to coordinate as a trianionic ligand. Under numerous conditions, the structures and properties of these compounds were characterized via absorption spectroscopy, X-ray crystallography, and NMR spectroscopy.

Andrew Dull – Biochemistry #6

Research Faculty Advisor: Adam Daly, Chemistry and Biochemistry

Calculations and Measurements of Biomolecular Analog Pyrithione with Formic Acid and Water

This study presents predicted structures and rotational spectra for the most stable complexes formed between pyrithione and both formic acid and water. These predictions are used as the basis for ongoing experimental efforts using microwave spectroscopy to detect gas-phase rotational transitions of the complexes. In the dimers formed between pyrithione and either formic acid or water, a combination of both hydrogen bonding and dipole-dipole interactions is expected to induce the greatest stability and tightest binding. The most stable pyrithione–formic acid complex reached an energy of –911.705 Hartrees with a binding energy of –6292.13 cm-1. Similarly, the lowest energy pyrithione–water complex showed an energy of –798.313 Hartrees and a binding energy of –4029.20 cm-1. Pyrithione serves as a possible analog for the pyrimidine groups found in nucleobases, providing insight into hydrogen-bond interactions between DNA and small organic molecules. While this work primarily focuses on computational modeling and calculations, future studies will delve further into experimental testing of these complexes.

Aranea Dunckley – Biochemistry; Molecular & Cellular Biology #36

Research Faculty Advisor: Marielle Hegetschweiler, Chemistry and Biochemistry

Exploring Hsp10 and its Interactions with Different Substrates

Heat shock proteins (Hsps) play a vital role in maintaining cellular homeostasis under stress conditions. Hsp10 is the co-chaperone of its much more studied chaperone counterpart, Hsp60. This chaperone machine plays a critical role in maintaining cellular homeostasis by folding unfolded or misfolded nascent proteins and preventing protein aggregation. Although previously considered a passive co-chaperone to Hsp60, recent evidence suggests that Hsp10 plays a more active part in substrate recognition and interaction. This research investigates the

interaction of Hsp10 with various substrate proteins, exploring its activity, binding kinetics, and functional implications. Through the use of a malate dehydrogenase (MDH) refolding assay and Microscale Thermophoresis (MST) experiment, we have put together a picture of Hsp10's novel importance in maintaining protein homeostasis. We show that Hsp10 alone, in absence of Hsp60, is active and promotes the folding of its natural substrate, MDH. Our results expand the understanding of Hsp10's role in cellular proteostasis and highlight its potential as a therapeutic target for diseases associated with protein misfolding and aggregation, including many neurodegenerative disorders.

Sam Ellis – Biochemistry #18

Research Faculty Advisor: Michael Taylor, Chemistry and Biochemistry

New Chemical-Proteomic Platforms for the Exploration of Mitochondrial Dynamics

The Taylor group has developed a pyridinium based molecular probe which can transfer a carbamate to tryptophan residues through photo-induced electron transfer (PET). By labeling and identifying reactive tryptophan residues, we can learn more about the proteins being labeled. This project aims to modulate an existing pyridinium based Cyclosporin A (CsA) probe. This probe allows for site specific protein modification as the CsA directs the molecule to the mitochondria. By synthesizing a similar probe with an extended linker between the CsA and the probe, new reactivity around the mitochondria may be found. The synthesis involves four steps using Grubbs metathesis to couple CsA to an amine containing molecule, followed by amide coupling to insert the polyethylene glycol (PEG) linker between the CsA and the pyridinium probe. Although the final product has not yet been synthesized, several products along the pathway have been isolated and identified.

Felicia Escalante – Biochemistry; Molecular & Cellular Biology #45

Research Faculty Advisor: Luciano Matias Matzkin, Entomology

Functional analysis of sperm competition of Drosophila arizonae

I determined the level of sperm competition between ARI11629 knockout males and a wildtype male, accounting for second male preference, where the last male to mate with a female sired most of her offspring. Virgin flies were collected, sexually matured, and sequentially mated with both wildtype and knockout males to assess sperm competition and paternity outcomes.

Our results demonstrate a strong second male preference effect in Drosophila arizonae, with the second male consistently siring more offspring. ARI11629 knockout males fail to fully benefit from this advantage, suggesting potential fertility problems.

Libby Farmer – Biochemistry #42

Research Faculty Advisor: Brian Enquist, Ecology and Evolutionary Biology

Reevaluating the Growth Rate Hypothesis: temperature stress, nutrient constraints, environmental variability, and stoichiometric flexibility

The Growth Rate Hypothesis (GRH) proposes that faster-growing organisms require higher phosphorus content due to the increased demand for ribosomal RNA to sustain rapid growth. While widely studied in invertebrates under constant conditions, the GRH's applicability across taxa under varying environmental conditions remains uncertain. Growth is influenced not only by temperature, which modulates metabolic processes, but also by nutrient availability and ecological constraints. High-temperature stress can disrupt cellular homeostasis and alter phosphorus allocation, yet some evidence suggests that rapid growth at elevated temperatures is maintained through enhanced protein synthesis efficiency rather than increased ribosomal RNA content, challenging the GRH's core predictions. Furthermore, Liebig's Law of the Minimum suggests that growth is constrained by the most limiting resource, meaning that phosphorus demand may not always be the primary determinant of growth,

especially under fluctuating environmental conditions. This study systematically reviews literature across taxa to assess the interactions between temperature, nutrient dynamics, stoichiometric flexibility, and environmental variability, evaluating whether the GRH holds under variable conditions. Findings demonstrate that the GRH fails to account for the combined effects of temperature variation, nutrient limitation, environmental variability, and stoichiometric flexibility. To address these gaps, a refined framework is proposed that integrates these factors, providing a more comprehensive approach for testing the GRH across diverse taxa.

Jacob Fredman – Biochemistry #12

Research Faculty Advisor: Robin Polt, Chemistry and Biochemistry

The Building Blocks of Glycopeptides

Peptide drugs are a quickly growing class of molecules with many hopes to provide new treatment options for various conditions. However, two major challenges in their development include aspartimide formation during solid-phase peptide synthesis (SPPS) and poor blood-brain barrier (BBB) permeability. This project presents two synthetic strategies to address these limitations. First, we synthesized a 2,4-dimethoxybenzyl (DMB)-protected aspartate-glycine (DG) dipeptide to suppress aspartimide formation under basic conditions. Second, we developed β-serine glucoside and β-serine lactoside building blocks by indium tribromide-catalyzed glycosylation, allowing for future incorporation into glycopeptide therapeutics that will allow for significantly increased BBB permeability. The purity and identity of all products were confirmed by ^{1}H NMR and HPLC analysis. This project offers a strong foundation for future development of peptide drugs.

Moorea Gailloux– Biochemistry #55

Research Faculty Advisor: Melanie Hingle, Nutritional Science & Wellness

Clinical Impacts of "Food is Medicine" on Hemoglobin A1c for Patients with Type 2 Diabetes

Type 2 Diabetes Mellitus (T2DM) is a chronic disease associated with elevated hemoglobin A1c (HbA1c) levels, increasing the risk of cardiovascular complications and mortality. HbA1c is a clinical marker reflecting average blood glucose levels over a three-month period, with reductions of ≥1% considered clinically meaningful. Evidence shows that food insecurity exacerbates poor glycemic control, particularly among low-income populations. In response, Food is Medicine (FIM) interventions aim to improve access to nutritious foods and provide dietary support to manage chronic conditions like T2DM. The objective was to assess the potential for FIM interventions to produce clinically significant decreases in Hba1c among persons with T2DM, thereby decreasing risk of future cardiovascular complications and morbidity. A rapid systematic review was conducted following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and PICO framework. The initial database search resulted in 5,665 studies. After the three-step screening process, 15 quantitative interventions met the inclusion criteria. Of these, six studies reported statistically significant reductions in HbA1c post-intervention. Two studies– Veldheer et al. (-1.3%, p<0.001) and Ferrer et al. (-3.9%, p=0.012)–reported both statistically and clinically significant decreases in HbA1c.These findings suggest FIM interventions may support clinically significant improvements in HbA1c through consistent access to healthier foods and diabetes self-management education. Future FIM programs should be implemented with rigorous, randomized designs, larger sample sizes, and timely follow-up assessments to strengthen the evidence base and inform policy and healthcare practice.

Melanie Galvan Salazar – Biochemistry; Molecular & Cellular Biology #13

Research Faculty Advisor: Robin Polt, Chemistry and Biochemistry

Glucose Glycosylation via ZnI_2: A Route to Modified PACAP

Synthesis of glycopeptide drugs related to the neurohormone PACAP (Pituitary Adenylate Cyclase-Activating Polypeptide) and other endogenous peptides begins with a glycosylated amino acid. Using previously optimized methods for glycosylation of alpha amino acids with minimally competent Lewis acids like InBr3 and Bi(OTfl)3, ZnI2 was found to have similar catalytic effects. Multiple attempts with various solvents, reaction times, stoichiometry, work up procedures, and purification steps were able to produce reduced amounts of product compared to procedures using InBr3. Although there was still some catalytic activity from ZnI2, it was found to be difficult to store due to its sensitivity to light, high reactiveness to the atmosphere, and extra steps were required to ensure complete removal from the end product. Overall, it's catalytic properties were successfully proven, but this salt was not as effective as InBr3 and Bi(OTfl)3.

Mary Gordon – Biochemistry #54

Research Faculty Advisor: Ashley Snider, Nutritional Science & Wellness

The Role of Dietary Fat in Modulating ER Stress and Inflammation in Ceramide Synthase 5/6 Knockout Mice

With the increased prevalence of processed and high fat foods in the "western" diet, there has been an increase in colitis and colorectal cancer. Endoplasmic reticulum (ER) stress has been implicated in diseases such as colitis and Crohn's Disease. Dietary fat and specific fatty acids have been implicated in regulating ER stress in the intestines. Ceramide, the central bioactive lipid in sphingolipid metabolism, is generated by six ceramide synthases (CerS). Ceramides have been shown to induce ER stress in intestinal epithelial cells. CerS5 and 6 are involved in incorporating C14:0 (myristate) and C16:0 (palmitate) saturated fatty acids into the acyl-chain of ceramide. In this study, mice that had both CerS5 and 6 knocked out in intestinal epithelial cells were placed on a milk fat based, lard based, or control diet. The different diets have different lipid compositions, which helps us to understand how different types of fat influence ER stress. Colon and ileum samples were collected from mice after 16 weeks of the diet. qPCR and western blot analysis were used to define the impact of specific high fat diets on ER stress in the intestines. Mice that were fed a milk fat based diet, which is high in myristate, showed increased inflammation and ER stress compared to those fed the lard based or control diet. Loss of CerS5/6 also increased ER stress in mice fed the milk fat diet. Knockout mice on the milk fat based diet also had higher expression of lipid metabolism markers in the colon. This trend was not measured in the ileum suggesting that diet-induced ER stress is focused in the colon. Future directions include the examination of specific segments of the small intestine to determine if ER stress increases in the duodenum or jejunum. Investigation as to the impact dietary fatty acids in intestinal ER stress, inflammation and lipid metabolism may provide insight into understanding the impact of diet in colitis and its causes.

Madison Grams – Biochemistry #20

Research Faculty Advisor: Elisa Tomat, Chemistry and Biochemistry

PH3, a redox active chelator and its effect on breast cancer cells, MDA, and ovarian cell cells, A2780

Iron is integral to cancer growth therefore iron deprivation can induce cell death in malignant cells. In addition, the redox chemistry of iron can lead to heightened oxidative damage through the Fenton reaction. This investigation looks at a new type of iron prochelator that is hypothesized to cause elevated oxidative damage and results in increased concentrations of reactive oxygen species (ROS), which are detrimental to the cell. Two different cancerous cell lines were treated with the prochelator and its glycoconjugate analog: tests were conducted in MDA-MB-231 (MDA) breast cancer cells and A2780 ovarian cancer cells, and the results were compared to MRC5 normal fibroblasts.

The disulfide-based iron prochelator PH3 was found to generate ROS when bound to iron, both in vitro and intracellularly. The treatment of MDA breast cancer cells with the antioxidant N-acetylcysteine (NAC) and PH3 did not lead to the anticipated cell rescue but instead resulted in increased toxicity. To understand this increased toxicity, the redox state of the cells was assessed using a fluorescent probe to detect ROS. The assay revealed that NAC reduced ROS levels, suggesting that oxidative damage was not the cause of the increased toxicity. UV-visible absorption and mass spectrometry data showed that NAC reacts with PH3 to form a bioconjugate that enhances cellular uptake. An iron-binding fluorescent probe was utilized to estimate the relative amount of PH3 taken up by the cells with or without NAC. The conjugate was subsequently synthesized and isolated to assess its antiproliferative activity and confirm its role as the active species in solution.

Further studies in ovarian cancer cells showed that PH3 binds intracellular iron and alters the expression of the iron storage protein ferritin H and the transferrin receptor TFR1. AH1 and its glycoconjugate G6AH1 were also examined, alongside PH3 and G6PH3 to demonstrate their greater toxicity to A2780 cells compared to noncancerous MRC5 cells, thereby highlighting their therapeutic index. Fluorescence-based assays showed increased production of ROS in cells treated with PH3. The toxicity of the iron complex of PH3 was found to depend on intracellular glutathione levels. Additionally, a glycoconjugate strategy was employed to enhance the targeting of cancer cells via the glucose transporter GLUT1, which is overexpressed in many cancer types. It is anticipated that combining a pro-oxidant chelator with a carbohydrate moiety will yield highly selective cytotoxic agents for tumors.

Alyssa Gregory – Biochemistry; Microbiology; Nutritional Sciences, Physiology & Medical Sciences #57

Research Faculty Advisor: Liliana Salvador, Animal/BioMed Science

Understanding tuberculosis dynamics in a wildlife species using phylodynamic approaches

Bovine tuberculosis (bTB), caused by Mycobacterium bovis, remains a persistent challenge at the wildlife-livestock interface, particularly in Michigan, where white-tailed deer (Odocoileus virginianus; deer) serve as a reservoir for transmission to cattle. Understanding the evolutionary dynamics and transmission patterns of M. bovis in deer is critical for developing targeted control strategies in this host-species. In this study, we focus on the role of sex and age in the transmission and persistence of M. bovis in deer. We use whole-genome sequencing data and phylodynamic approaches to analyze 626 M. bovis-positive deer samples extracted from deer. We estimate genetic distances and construct maximum-likelihood phylogenies with bootstrap support using R, and assess temporal structure using TEMPEST. Phylogenetic analyses in the Bayesian Evolutionary Analysis Sampling Trees (BEAST) program are used to determine the evolutionary rate of M. bovis, estimate the time of the most recent common ancestor (TMRCA) of M. bovis strains in Michigan deer, and infer transmission patterns through Discrete Trait Analysis (DTA) based on sex and age classifications. Our research provides deeper insights into the transmission dynamics of M. bovis in deer, informing more effective surveillance and control measures to mitigate bTB spread at the wildlife-livestock interface.

Megan Hahn – Biochemistry #14

Research Faculty Advisor: Jeffrey Pyun, Chemistry and Biochemistry

Synthesis and Functionalization of Magnetic Nanoparticles with polymer brushes for magneto-optical photonics

The development of stable magneto-optically active nanocomposites has gained significant traction over the past decade for advanced optical and magnetic sensing applications. Magnetic nanoparticles (MNPs) are widely used in MO nanocomposites due to their tunable magnetic properties, nanoscale dimensions, and high surface area. When embedded within a polymer matrix, these nanoparticles can be effectively dispersed and stabilized, enabling the design of materials with enhanced magnetic responsiveness and optical clarity. However, challenges such as particle aggregation, chaining, and maintaining transparency at higher inorganic loading can arise within

these complex matrices. In this work, we present the optimization of generating stable MNP nanocomposites with tunable properties, such as the Verdet constant, achieved by controlling factors like particle size and inorganic content loading. The manipulation of particle size is achieved through various methods, including varying reaction temperature, changing metal-to-ligand ratios, and adjusting surface ligand molecular weight, all of which influence the growth rate and final dimensions of the nanoparticles. While increasing particle size can lead to chaining which induces light scattering, we employ controlled radical polymerization (CRP) techniques to achieve uniform interparticle spacing, thereby enhancing transparency and overall performance. This research contributes to the development of high-performance MO materials suitable for next-generation photonic and sensor technologies.

Olivia Hajdys – Biochemistry; Molecular & Cellular Biology #38

Research Faculty Advisor: Pascale Charest, Molecular and Cellular Biology

Investigating cAR1 interactions in Dictyostelium Discoideum

Metastatic cancer is the most advanced, aggressive stage of cancer, and unfortunately, it is the most deadly form of cancer. While there are treatments that can help slow tumor growth and treatments that can ease symptoms, there is no guaranteed cure for metastatic cancer. The model organism dictyostelium discoideum, also known as a social amoeba, is studied to discover more about the cellular pathways of metastatic cancer. This social amoeba has a highly conserved chemotactic signaling pathway in mammalian cells, which uses cyclic AMP (cAMP) to guide the development pathways. Research has previously found that cyclic AMP receptors (cARs) are G-Protein Coupled Receptors (GPCR) which respond to cAMP to initiate the chemotaxis pathway. Research has also shown that Calmodulin A (CalA) is a calcium cation dependent signaling protein that is recruited to cAR1, among many other proteins, during the chemotactic signaling processes. We want to find out if CalA is involved in cAR1 activity during chemotaxis, as cAR1 has calmodulin binding sites. In order to determine this, we want to use Bioluminescence Resonance Energy Transfer (BRET) by fusing Green Fluorescent Protein (GFP) to a CalA construct and fusing a luminescent molecule, LuciferaseII (LucII), to the C-terminus of cAR1. If the two constructs interact, a BRET ratio will be observed that supports the excitation of GFP via the donor emission of LucII.

Rhett Hill – Biochemistry; Molecular & Cellular Biology #7

Research Faculty Advisor: Adam Daly, Chemistry and Biochemistry

Microwave spectrum and molecular structure calculations for a butadiene iron tricarbonyl–water complex

The first gas phase microwave spectra for a dimer between water and an organometallic complex were measured using a pulsed-beam Fourier transform microwave spectrometer. 32 rotational transitions for the weakly bound complex of butadiene iron tricarbonyl and water were measured in the region of 6-14 GHz. These transitions were analyzed and fit to determine rotational constants of A = 973.9279(9) MHz, B = 606.1943 (4) MHz, C = 605.2675 (4) MHz. Computational studies were performed on the University of Arizona PUMA-9 using Density Functional Theory (DFT) and MP2 methods. G-16 calculations identified two stable low-energy structures. In Structure A, the water molecule is hydrogen-bonded to the methylene side of the butadiene complex, whereas in Structure B, the water is positioned on the opposite side of the methylene group. Structure A, calculated using B3LYP/aug-cc-pVTZ Empirical Dispersion=GD3 provides v=0 rotational constants in excellent agreement with measured values. There appear to be 3 hydrogen bonds stabilizing the dimer.

Carter Hollings – Biochemistry; Molecular & Cellular Biology #62

Research Faculty Advisor: Kai Staats, Biosphere

Analysis of Relative Microbial Abundance and Growth in Short Term Martian Colony Simulation Utilizing 16s rRNA Amplicon Next-Generation Sequencing

The study of microbial communities in enclosed environments is critical for understanding the challenges of both short and long-duration space missions, especially with emphasis on those with habitats beyond Earth. This study seeks to realize the shifts in surface microbes inside a short term simulated Martian colony at the Space Analogue for the Moon and Mars (SAM). Samples of bacteria and archaea came from 12 distinct surfaces and were taken 10 days apart. These samples were then taken and next generation sequencing was performed on them. While the data has yet to be finalized, this work sets a guide to learn how microbes change in closed environment, off-world setups. Based on the samples we took, the diversity and density of microbes exploded.

Lily Jensen – Biochemistry; Molecular & Cellular Biology #8

Research Faculty Advisor: Adam Daly, Chemistry and Biochemistry

Calculated Microwave Rotational Spectrum of 2-Aminopyridine Complexes: Predicted Spectra, Structures, and Rotational Constants

2-aminopyridine is a molecule that acts as an analog for the DNA base adenine. Studying the rotational spectrum of this molecule can provide insight into the intramolecular interactions of DNA bases, which are difficult to analyze. This project uses microwave rotational spectroscopy to measure rotational spectra and structure of small gas phase molecules. High-level calculations using density functional theory (DFT) methods and various basis sets were performed to predict rotational constants, quadrupole coupling constants, and frequencies of rotational transitions. The main objective of this project was to use the technique of microwave spectroscopy to determine the molecular structure of 2-aminopyridine when interacting with other small organic molecules. Future research can be done to determine the structure of complexes of 2-aminopyridine and other DNA base analogous structures with small organic molecules that may interact with DNA.

Dylan Kmiec – Biochemistry #4

Research Faculty Advisor: Minying Cai, Chemistry and Biochemistry

Investigating PACAP Truncations and Receptor Interactions Using Plasmon Waveguide Resonance Spectroscopy

Pituitary adenylate-cyclase-activating polypeptide (PACAP) is a neuropeptide critical for numerous physiological functions, including neuroprotection, vasodilation, and pain modulation. This research aimed to characterize the structural and biophysical properties of truncated PACAP peptides through experimental analysis using plasmon waveguide resonance spectroscopy (PWR). PWR provided real-time, quantitative data on the binding interactions of truncated PACAP variants with lipid membranes and receptor fragments, offering insights into their affinity, conformational behavior, and functional potential. Experimental data revealed significant variability in receptor affinity and conformational changes among truncations, particularly highlighting the modulatory role of glucose conjugation on binding strength and receptor specificity. The findings emphasize critical residues influencing receptor binding and demonstrate pronounced differences in affinity between receptors VPAC1, VPAC2, and PAC1. These insights provide foundational knowledge for the design and optimization of peptide-based therapeutics targeting disorders involving GPCR dysregulation.

Seth Larger – Biochemistry; Molecular & Cellular Biology #28

Research Faculty Advisor: Tarjani Thaker, Chemistry and Biochemistry

Defining the molecular basis for transmembrane domain interactions in COQ8B complexes

Coenzyme Q (CoQ) plays a crucial role in various biological processes, most notably in oxidative phosphorylation, where the hydrophobic, lipid-like molecule helps transport electrons through the electron transport chain to produce ATP. Imbalances in CoQ levels are associated with several diseases, including cardiovascular disease, kidney disease, and certain cancers. CoQ is synthesized by a group of proteins in the mitochondria, forming a biosynthetic hub called Complex Q. One of these proteins, the ABC1 family transmembrane kinase CoQ8B, is implicated in the final stages of CoQ production, but its exact role remains unclear. Several disease-associated mutations in the transmembrane domain of COQ8B suggest that this domain may help stabilize COQ precursors or other components of Complex Q during CoQ synthesis. In this study, I investigate the role of the transmembrane domain of CoQ8B in its structure, function, and regulation. Our preliminary findings from thermostability experiments on wild-type and mutant complexes of COQ8B reveal a role for a conserved dimerization motif in complex stability. Future experiments will entail investigating the relationship between dimerization and kinase function in COQ8B.

Ika Lin – Biochemistry #10

Research Faculty Advisor: John Jewett, Chemistry and Biochemistry

Exploring Caged Aryl Diazonium Ions as Fluorogenic Probes

Tailored chemical probes are essential to targeting and reporting on elements of complex biological systems. While fluorescent probes can provide key spatial information regarding biomolecules of interest (BOI), they can suffer from low signal to noise ratios and encounter specificity issues due to bioaccumulation. Fluorogenic probes present a compelling alternative in that no fluorescence is emitted until the probe is bound to or activated by the BOI, offering additional functional utility. Previous work in the Jewett Group has established protected triazabutadienes (TBD) as effective intracellular delivery mechanisms for highly reactive aryl diazonium ions (ADI) upon protonation. This project aims to functionalize the TBD scaffold with protection groups sensitive to environmental and/or enzymatic conditions. Once the TBD is fully deprotected in the presence of these conditions, the released ADI will cyclize to form a fluorescent benzocinnoline (CinBen) probe. Since each logic-gated outcome from protected TBD to released CinBen will result in distinct fluorescence excitation and emission spectra, or lack thereof, this enables quantifiable detection of the probe's real-time state. To this end, a pro-fluorogenic TBD has been synthesized. The structures of the TBD and resulting CinBen were characterized by NMR and fluorimetry. While the TBD is fluorescent, it absorbs and emits light at different wavelengths than the CinBen probe. Our next steps will involve protecting the TBD at two positions to better control its reactivity. Further work will yield applications for in vivo imaging and enzymatic assays, offering greater insight into biomolecular functions within complex systems.

Melvin Lopez – Biochemistry #27

Research Faculty Advisor: Matthew Cordes, Chemistry and Biochemistry

The role of a non-catalytic lipid binding site in substrate recognition by recluse spider toxins

Venom from recluse spider toxins is known to cause dermonecrotic lesions and other systemic effects on humans who are inadvertently bitten. Phospholipase D (PLD) toxins that bind and attack membranes are responsible for venom potency in humans and prey. Phylogenetic analysis of toxin genes shows variable lipid substrate preference of two major clades: the α clade toxins showing preference for choline head groups and β clade toxins showing preference for ethanolamine head groups. Substrate lipid preference of PLD toxins can be attributed to the structure of the active-site head-group binding pocket. Yet, α clade toxins are known to contain sites outside the active site significant to choline head group recognition, suggesting that surface or allosteric activation has coevolved to support active site preference of choline. To test headgroup recognition through non-catalytic sites,

we performed site-directed mutagenesis on a recently identified non-catalytic site in a β clade protein (Stβ1B1i) to mimic that of an alpha clade protein (Liα1A1). Enzyme-coupled spectrophotometric assays on Stβ1B1i T225Y/S233T revealed decreased activity towards ethanolamine substrates but no observable increase in activity towards, or allosteric activation by, choline substrates. Liposome binding assays showed no increase in binding to the choline substrate sphingomyelin. We conclude that this mutation in the non-catalytic site decreased activity toward ethanolamine substrates but showed no evidence of increased interaction with choline substrates. It is possible that this pocket in β clade enzymes has the potential to only bind to ethanolamine and not choline head groups.

Joshua Mahar – Biochemistry #16

Research Faculty Advisor: Michael Taylor, Chemistry and Biochemistry

Synthesis of photoreactive pyrdidinium and pyrimidinium molecular probes for the purpose of protein labeling

Photo-reactive molecular probes are an important field of research, as molecular probes allow for direct covalent modification of protein-residues that can then allow for identification. This identification can be used to help measure protein levels in cancerous cells, as the amount of covalently modified proteins in cancer cells can then be compared to the modified protein levels of non-cancerous cells. The molecular probes that we have synthesized contain pyridinium and pyrimidinium groups, with a light sensitive N-N bond, that when shined on by a wavelength of light of 456 and 475 nm respectively. We lay out the synthesis of these probes and demonstrate how Pyridinium and Pyrimidinium based probes are capable of labeling lysozyme proteins under varying pH levels.

Binay Maharjan – Biochemistry #35

Research Faculty Advisor: Thomas Tomasiak, Chemistry and Biochemistry

Disulfide Glass: Identification and Characterization of the Catalytic Site in Candida auris FKS1 Using Computational Modeling

On earth there exist a whole myriad of infections in our world, they range from virus, bacterial all the way to fungal infections. In these decisions of the different types of infections that there in the world to this point there is a focus in regard to this lab in line with fungal infections. The reason being they are unique in ways that bacteria and viruses are not in that they are eukaryotic. Making them special in terms that they are closer related to human and other animals than they are to bacteria prokaryotes. This causes fungal infection to be more dire and harmful to human health. Furthermore, fungal infections are more complex comparatively to the other infections from the cell structures, making it harder to deal with such infections. This alone makes fungal infections and the study of their structure very interesting as their classical term. In terms of this study is in regards Candida genus of fungal infections due to this fungal infection type is more harmful than others due to it reaction to antifungal medication and how resilient they are to them. This causes many more people to get sick as well as not being able to get better from medication. In specific on Candida, the species that is investigated heavy is Candida albican due to its dangerous fungal pathogen, where it heavily affects people who are immunocompromised and causes a range of severe infections. C. auris within has a protein Fks1 which is responsible for the fungal infection to have the ability to encode the cellular wall synthesis. This comes in line due to Fks1 is a gene that encodes a catalytic subunit of β-1,3- glucan synthase. The goal is to understand how Fks1 operates and how its activity can be inhibited. Mutations in FKS1 allow the fungus to survive even in the presence of antifungal drugs by reducing drug binding to its target. This research would lead to new treatments that can prevent C. aruis from resisting medication, in order to make solutions for immune compromised people from suffering.

Benjamin Maldonado – Biochemistry #11

Research Faculty Advisor: John Jewett, Chemistry and Biochemistry

Selective Protein Labeling at High pH Using Meldrum's Acid-Based MaMa Probes and SPAAC Chemistry: Toward Efficient and Metal-Free pH-Responsive Bioconjugation

Protein bioconjugation is a cornerstone technique in chemical biology, enabling targeted imaging and drug development. However, challenges such as probe instability, side reactions, and biological complexity often limit the effectiveness of many conventional strategies. In this work, we present a pH-responsive bioconjugation platform based on a Meldrum's acid amine-reactive Michael acceptor (MaMa) probe, designed for selective lysine residue modification in high-pH environments (pH ≥ 10). This MaMa system demonstrates improved stability and chemoselectivity over traditional lysine-targeting methods. To enable downstream labeling, a variant of the MaMa probe was developed to incorporate a bioorthogonal handle for copper-free click chemistry via strain-promoted azide–alkyne cycloaddition (SPAAC), using a bicyclo[6.1.0]nonyne (BCN) partner. Experimental validation through protein labeling assays, SDS-PAGE, and fluorescence imaging confirmed successful, site-specific protein modification under biologically relevant conditions. Robust labeling at pH > 9 underscores the utility of this approach for biochemical applications where traditional conjugation strategies fall short. Together, this work contributes a versatile and selective toolkit for stable protein bioconjugation in complex systems, with potential applications in imaging, diagnostics, and targeted delivery.

Ginelle Maldonado – Biochemistry #53

Research Faculty Advisor: David Margolis, Orthopaedic Surgery

Micro CT Analysis of Bone Regeneration in Critical Size Defects Using Polymer Scaffolds

Defects in long bones are a frequent global issue, often caused by trauma. While bone tissue has a natural ability to heal, large defects may require intervention to promote effective regeneration. This study investigates femur fracture healing in an in vivo sheep model, using intramedullary implant nails for varying durations and incorporating a polymer scaffold to enhance bone repair. Results showed that longer implant retention times led to greater bone growth around the defect. However, increased bone porosity was also observed, suggesting that while more bone formed, it was less dense and potentially weaker. Early time points revealed smaller areas of bone growth but with a denser and more organized structure, highlighting a trade-off between quantity and quality of regeneration. The polymer scaffold contributed to faster initial bridging of the defect, supporting early cell infiltration and matrix deposition, though integration varied over time, with some areas showing incomplete mineralization. Overall, the findings suggest that although prolonged implant presence and scaffold use can enhance bone volume, achieving optimal bone density remains a challenge. Future strategies may focus on improving scaffold design and regulating healing timeframes to promote both robust and high-quality bone regeneration.

Dilan Maliyagoda – Biochemistry #5

Research Faculty Advisor: Minying Cai, Chemistry and Biochemistry

Exploring Minimal Functional Pharmacophore of PACAP using Plasmon Waveguide Resonance Spectroscopy and Schrödinger

Peptide-based therapeutics offer a safer and more selective alternative to conventional small-molecule drugs. Thus, endogenous peptides have become a lucrative target for drug development. Here, we focus on one step of this process and look to identify the minimal pharmacophore of Pituitary Adenylate Cyclase Activating Polypeptide (PACAP). PACAP is an endogenous neuropeptide consisting of two 38 and 27 amino acid isoforms. PACAP signals through three G-coupled Protein Receptors (GPCR), PAC1, VPAC1, and VPAC2. PAC1 demonstrates a 1,000-fold higher affinity for PACAP vs VPAC1 and VPAC2. PACAP is believed to play a significant role in neural development, endocrine regulation, immune system function, and the protection of both gastrointestinal and

renal systems. Consequently, PACAP has emerged as a promising candidate for peptide-based drug development. The Cai Lab contributes to this initiative by employing an innovative approach to the classic pipeline of peptide-based drug development. Novel technologies such as Plasmon Waveguide Resonance Spectroscopy (PWR) are used to monitor receptor ligand interactions in order to determine a viable truncation of PACAP. In addition, we use Schrödinger, a molecular simulation program, to model the secondary structure and simulate ligand-receptor interactions for each truncation. These computational insights are integrated with experimental data to expedite the identification and validation of PACAP's minimal pharmacophore, achieving greater efficiency than traditional methods.

Luis Mendez Elizondo – Biochemistry #26

Research Faculty Advisor: M. Leandro Heien, Chemistry and Biochemistry

Use of metal oxides to improve reference electrodes biocompatibility and stability

One of the most important aspects of neurochemical analysis is taking accurate measurements in-vivo with a certain degree of stability but finding an ideal reference electrode that is biocompatible and stable enough to provide accurate measurements over time has proven difficult. The use of metal oxides is a promising solution to this problem. Here we explored different options to create reference electrodes ranging from coating tungsten and stainless-steel wires in a tungsten oxide coating though the use of pulse voltammetry applying a square waveform ranging from 2V to 4V, as well as stimulating an oxide film in an Iridium wire by oxidizing it in an aqueous disodium phosphate solution. While severe issues with the stainless steel and tungsten electrodes prompted us to discontinue experiments on them, IrOx showed the most promise by having a good stability and affinity to dopamine of 5.85 nA/mM. We were able to show that IrOx electrodes, particularly long ones, can be recycled and reused while retaining a stable OCP, which combining with storing them dry is more beneficial than storing them in aCSF since electrodes stored dry only had a change between -18.45% to -7.4% in the span of one week.

Gabriella Miranda – Biochemistry; Molecular & Cellular Biology #43

Research Faculty Advisor: Hillary Mehl, Plant Sciences

Expression of Polyketide Synthase by Aspergillus Flavus During Competition of Aflatoxigenic and Non-aflatoxigenic Genotypes

Aflatoxin B1 is a carcinogenic mycotoxin produced by Aspergillus flavus. Non-aflatoxigenic strains of A. flavus are used as biocontrol to reduce aflatoxin contamination in crops. Biocontrol isolates have mutations in their aflatoxin biosynthesis gene cluster causing an inactivation in one or more of the genes. The polyketide synthase gene (pksA) is active at the beginning of the aflatoxin biosynthesis pathway, and in this study the expression of pksA by AF13, an aflatoxigenic strain of A. flavus, was assessed under different conditions. The objectives of this study were to quantify the extent to which competitive exclusion and changes in gene expression influence aflatoxin production when an aflatoxigenic A. flavus interacts with different biocontrol genotypes, and to identify differences among biocontrol genotypes in how they influence expression of pksA, a critical gene for aflatoxin biosynthesis. AF36 is a biocontrol strain that is used in Arizona and has a single nucleotide polymorphism (SNP) in pksA that causes an early stop codon. FourSure is a multi-strain biocontrol product used in Texas that has four different non-aflatoxigenic genotypes (TC46G, TC16F, TC35C, and TC38B), each have variable SNPs and deletions in the aflatoxin gene cluster. AF13 was co-inoculated with different biocontrol strain genotypes in Czapek's broth, and changes in pksa expression, percent AF13, and aflatoxin concentrations were measured overtime. pksA changed overtime and was highest after 4 days. AF36 and TC16F did not significantly influence pksA, but reduced aflatoxin concentration through competitive displacement of AF13. In contrast, TC46G altered expression of pksA by AF13. Overall, the expression of pksa by AF13 varied depending on the biocontrol genotype it was competing with and the time point in the growth cycle of the competing A. flavus.

Adrian Moreno – Biochemistry #58

Research Faculty Advisor: Arun Dhar, Animal and BioMedical Sciences

Exploring alternatives to glacial acetic acid for the preparation of davidson's afa solution used for fixation of shrimp tissues

The growth and sustainability of shrimp farming worldwide relies on the detection and prevention of infectious diseases. Farmers rely on regular health assessments to detect diseases. Histopathology and PCR-based diagnostics are the main means of conducting health assessments within a laboratory setting but rely on adequate sample fixation at the farms. Davidson's Alcohol Formalin Acetic acid (DAFA) has been the gold standard for the preservation of shrimp tissues for histopathological analysis for half a century. However, in many regions around the world, chemical-grade glacial acetic acid (GAA) is difficult to acquire, expensive, and is difficult to ship due to its hazardous classification. To make shrimp sample preparation and shipping more accessible, we evaluated two possible alternatives for GAA in DAFA: 30% industrial-strength vinegar (ISV) and 50% citric acid (CA). These Alternatives were chosen because they are inexpensive and readily available worldwide due to their use as a household cleaner and a common cooking ingredient. For initial testing, healthy Penaeus vannamei shrimp were fixed with DAFA or a modified fixative where GAA was replaced with ISV or CA. The shrimp were then processed according to conventional techniques for paraffin embedding and sectioning. Sections were stained with Mayer-Bennett's hematoxylin/eosin- phloxine (H&E) and examined via light microscopy. Analysis revealed that the fixative modified with ISV provides the same quality of tissue preservation as traditional DAFA, indicating that it may be a viable alternative. The fixative modified with CA did not provide the same quality of fixation indicating that it would not be a viable alternative.

Alejandro Noriega – Biochemistry; Molecular & Cellular Biology #31

Research Faculty Advisor: Thomas Tomasiak, Chemistry and Biochemistry

Computational Modelling of Substrate Recognition by the Cdr1 Transporter in Candida auris: Implications for the development of novel antifungals agents

Candida auris is a growing global health concern, causing approximately 4 million deaths annually. With high levels of resistance against different anti-fungal drugs, C. auris poses serious public health concerns. This calls for the identification of molecular mechanisms underlying the drug resistance and discovery of new age anti-fungal drugs. Candida drug resistance 1 (Cdr1) protein, an ATP-binding cassette (ABC) transporter, is identified as one of the major players involved in drug efflux. A lot of research has been done on the drug efflux activity of Cdr1 in Candida albicans; however, how the structural and functional mechanisms involved in drug efflux activity of C. auris Cdr1 remain less explored. Here, in this study, we have taken the advantage of advanced computational tools like Alphafold3, Chai Discovery, and PaddleHelix to model the binding interactions of known Cdr1 substrates. Additionally, we employed similar computational strategies to identify potential binding sites for prospective Cdr1 inhibitors. Our findings offer structural and computational insights into the substrate-binding pocket of Cdr1, laying the groundwork for future experimental research aimed at antifungal drug development.

Ellie Osborne – Biochemistry #41

Research Faculty Advisor: Christopher Pappas, Cellular & Molecular Medicine

Investigating the Functional Role of Actin-Binding Site 1 (ABS1) in Leiomodin2 Regulating Thin Filament Elongation in Cardiac Muscle

Cardiac muscle contraction depends on the precise assembly of sarcomeres, particularly the regulation of thin filament length at their pointed ends. Leiomodin-2 (Lmod2), a cardiac-specific actin-binding protein, promotes actin polymerization at the pointed end, competing with the capping protein Tropomodulin-1 (Tmod1). Lmod2's actin-binding site 1 (ABS1), composed of a disordered N-terminal region and a C-terminal amphipathic α-helix, is

thought to regulate polymerization by acting as a “leaky cap.” Dysregulation of Lmod2, including mutations in ABS1, has been implicated in dilated cardiomyopathy (DCM), a disease that often leads to pediatric heart failure.

In this study, we investigated how specific mutations within ABS1 affect actin polymerization. Two mutants were generated: Lmod2-GG (N45G/L46G), targeting the disordered N-terminal half, and Lmod2-A1QM+GG (F64D/L69D/Y72D/W73D plus N45G/L46G), targeting both ABS1 regions. Proteins were purified and used in pyrene-actin polymerization assays to measure nucleation activity. Results showed that the Lmod2-GG mutation significantly increased polymerization rates compared to wild-type Lmod2, suggesting reduced actin-binding affinity and easier displacement during filament elongation. In contrast, the combined A1QM+GG mutant did not significantly differ from wild-type, possibly due to compensatory effects between increased and decreased binding affinity mutations.

These findings support a model in which ABS1 plays a critical role in controlling pointed-end dynamics and suggest that specific mutations may enhance filament elongation. This has implications for therapeutic approaches to DCM, where thin filament shortening impairs contractility. Future work will examine filament lengths and structural changes using GFP transduction and NMR spectroscopy to validate the functional impacts of these mutations.

Gary Palmeri – Biochemistry #49

Research Faculty Advisor: Patrick Ronaldson, Pharmacology

Acetaminophen’s Effect on Blood-Brain Barrier Transporters: Expression of P-gp and BCRP in the Rat Cortex, Cerebellum, Hippocampus, and Thalamus

Acetaminophen (APAP) is a widely used analgesic, yet its influence on the blood-brain barrier (BBB) and drug transport mechanisms remains unclear. This study investigates the effects of APAP on the expression of two major BBB efflux transporters—P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP)—across four brain regions: the cortex, cerebellum, hippocampus, and thalamus. Male Sprague Dawley rats were treated with either vehicle or 80 mg/kg of APAP. Brain microvessels were isolated, and protein expression levels were quantified using Western blot analysis. Preliminary results indicate regional differences in transporter expression, with slight increases in P-gp and BCRP in some APAP-treated samples, although these changes were not statistically significant. Current work is underway to assess the functional impact of these findings by measuring ketamine accumulation in the brain via liquid chromatography/mass spectrometry (LC/MS). This research aims to better understand APAP’s role in modulating BBB transporter activity and its potential implications for central nervous system drug interactions.

Javier Perez – Biochemistry #52

Research Faculty Advisor: Nathan Cherrington, Pharmacology

The Effects of PFAS Inhibition of Mouse Oat5 and Human OAT4 Transporters within Chinese Hamster Ovary Cells

Polyfluoroalkyl substances (PFAS) exposure into the human body has been researched and is known to cause a multitude of health related issues. In this study, the comparison of various PFAS inhibition was tested on human OAT4 and mouse Oat5 transporters expressed in Chinese Hamster Ovary (CHO) cells. A quantitative analysis of inhibition from 7 different PFAS molecules were conducted by radioactivity counting and shown in various IC50 graphs. From these results, PFAS do indeed inhibit and block the organic ion transporters expressed in mice and human. Understanding the effects of PFAS inhibition on organic anion transporters can provide further understanding of exposure to PFAS, and its mechanisms leading to the disposition from the body. It can also help us understand the chemical risks for future use of PFAS products. Furthermore, this study further supports the use of mouse Oat5 as the functional ortholog of human OAT4 in animal studies. This can help validate the use of mouse Oat5 as it compares it to human OAT4 for pharmaceutical studies and drug tests.

Imani Ralph – Biochemistry #25

Research Faculty Advisor: M. Leandro Heien, Chemistry and Biochemistry

Micrometer Chronic Electrode Development for Dopamine Detection

Dopamine serves as an important neurotransmitter to many biological functions and is the root for specific disorders, like Parkinson's Disease. Micrometer chronic electrodes allow for long-term detection of dopamine within the brains of mobile rats, which is a limitation of their acute electrode counterparts. The methodology for developing micrometer chronic electrodes becomes better defined for the purpose of reproducibility, evaluating the electrodes to ensure sufficient results can be obtained for dopamine related experiments. Through Electrochemical and Flowing Injection analysis, the adequacy of the micrometer electrodes' construction methodology can be analyzed through quantitative data and comparison to acute electrodes.

Dillan Rhodes – Biochemistry #48

Research Faculty Advisor: Richard Simpson, Nutritional Science Wellness

The Effects of Exercise and Recovery Serum on Growth Kinetics of Hematologic Malignancies

Epidemiological studies consistently show that exercise lowers incidence rates of several different types of cancer. Physiologically, there are several mechanisms that may underlie the anti-cancer effect of exercise including epinephrine dependent mobilization and redistribution of immune cells, normalization of blood flow in the tumor microenvironment and the release of systemic circulating factors. Exercise leads to the release of myriad circulating factors such as epinephrine and immune regulatory cytokines such as IL-6, 7, 15, and TNFa. In the context of cancer, such factors contribute a variety of systemic and local effects on metabolism and the immune system; presenting with a correlation in intratumoral immune cell infiltration depending on the intensity and duration of physical activity and may provide significant contribution to T cell variation and concentrations. In-vitro studies have shown that serum collected during and following acute bouts of exercise inhibits of cancer cell proliferation and lowers viability. While current literature suggests significant inhibition of cancer cell survival following exercise induced serum supplementation, investigations on hematologic malignancies are rare. In this study we aim to quantify the effect of circulating factors released during acute bouts of exercise on the growth kinetics of several hematologic malignancies. Following incubation with media supplemented with human sera from rest, exercise, or one hour post recovery timepoints, cell counts were normalized to FBS supplemented media and expressed as a fold change. All human serum supplemented media slowed the growth of the tumors when compared to FBS. Exercise and one hour recovery serum had an inhibitory effect on K562 tumor growth compared to media supplemented with resting serum. FBS normalized cell growth in the exercise and one hour post recovery conditions were ~20% lower than rest after one day in culture. [p = 0.0336, 0.0035, fold count mean difference: 19.00, 13.09]. Cells in exercise and one hour recovery conditions continued to exhibit a 25% delay in growth relative in the first 6 hours after serum refresh on day two. [p = 0.0025, 0.0097, fold count mean(s): rest = 86.33, exercise = 75.89, 1 hour post = 72.93]. There was no notable effect of serum supplementation on JEKO-1 tumor growth. In conclusion, these results demonstrates that exercise and recovery serum can inversely regulate cancer cell proliferation in a subset of hematologic malignancies.

Natalie Sanchez – Biochemistry #32

Research Faculty Advisor: Thomas Tomasiak and Darpan Raghav, Chemistry and Biochemistry

Substrate Selectivity of Candida auris Cdr1: Comparative Analysis of Short- and Long- Tailed Azoles via In Silico and In Vitro Approaches

Candida auris (C. auris) is a newly emerging fungal pathogen that is resistant to multiple drugs and presents a serious public health concern in the United States, especially in the healthcare settings. In 2023, the Centres for Disease Control and Prevention (CDC) reported 4,514 new clinical cases of C. auris in the U.S., marking a continued increase since the first reported case in 2016. C. auris is difficult to eradicate particularly due to its

growing resistance to current antifungal drugs. The Candida drug resistance 1 (Cdr1) protein is known to play a key role in expelling antifungal drugs, thereby contributing to drug resistance in Candida species. Azole antifungal drugs like fluconazole, ketoconazole, itraconazole, and posaconazole are well established substrates for Cdr1. Although all these drugs contain a five-membered heterocyclic azole ring, they differ in tail sizes. In this study, we combine computational modelling and in vitro experiments to investigate the molecular mechanisms underlying the selective interactions of short and long-tailed azole antifungals with Cdr1. We have successfully overexpressed Cdr1 from C. auris in the S. cerevisiae INVSc1 strain and purified it to a high degree of purity. Results from molecular docking and fluorescence polarization assay indicated that fluconazole (short-tailed azole) and posaconazole (long-tailed azole) got bound to the same cavity in Cdr1. Results from molecular dynamics simulations gave insights into the mechanisms by which Cdr1 differentiates and accommodates short and long tailed azoles in the cavity and expels them in the extracellular environment.

Lucas Schomburg – Biochemistry #33

Research Faculty Advisor: Thomas Tomasiak, Chemistry and Biochemistry

IMIDs Disrupt Hydrogen Bonding in the MCT1–CD147-CRBN complex: A Structural Analysis

Monocarboxylate transporter 1 is a ubiquitously expressed transporter that transfers lactate and pyruvate across the plasma membrane of cells. The glycoprotein, CD147, forms a complex with MCT1 and is a necessary chaperone for trafficking MCT1 to the membrane. This complex is important for the growth of tumor cells in multiple cancers, where it primarily imports lactate to drive glycolysis. Cereblon (CRBN) is a substrate receptor that is involved with the stabilization of the MCT1-CD147 complex. Immunomodulatory drugs (IMIDs) have been shown to outcompete CRBN for binding to MCT1 and CD147 which leads to the destabilization of the MCT1-CD147 complex. This paper analyzes MCT1 bound to CD147, CRBN and three immunomodulatory drugs: thalidomide, pomalidomide, and lenalidomide. This was done by generating AI predicted structures through the algorithm Chai-1. Multiple sequence alignments were also made for the proteins of interest. Through the structural analysis tools in ChimeraX, the residues HIS 378 and ASN 351 within CRBN, along with MET 1 within MCT1 were found to behave in interesting ways based on the presence or absence of an IMID. It is important to note that in most proteins MET 1 is cleaved off in the post-translation process, however, the time in which this happens in the context of the MCT1-CD147-CRBN interaction has not been studied. It appears that the presence of an IMID disrupts hydrogen bonding between MET 1 and HIS 378, ASN 351. These bonding interactions could open up a new pathway of disrupting the formation of the MCT1-CD147 complex.

Olivia Seagraves – Biochemistry #3

Research Faculty Advisor: Minying Cai, Chemistry and Biochemistry

Direct Biased Signaling Study of G protein-coupled receptor (GPCR) & Beta-Amyloid (Aβ) Kinetics Studies via Plasmon Waveguide Resonance (PWR) Spectroscopy

Biased agonism is increasingly leveraged in drug development by designing ligands that selectively activate specific intracellular signaling pathways downstream of a receptor. This approach enables targeted therapeutic effects while minimizing adverse side effects by avoiding the activation of undesired signaling pathways. In the context of opioid pharmacology, many of the adverse effects—such as respiratory depression, constipation, and physical dependence—are mediated by specific intracellular signal transducers. Drugs that induce biased signaling offer a promising strategy for enhancing therapeutic efficacy while mitigating these undesirable effects. Plasmon Waveguide Resonance (PWR) spectroscopy is a novel, label-free biophysical technique that enables real-time monitoring of molecular interactions and conformational changes using both p- and s-polarized light. PWR can be employed to directly investigate biased signaling pathways between ligand-bound human opioid receptors and intracellular transducers, providing insights into receptor dynamics with high sensitivity. In this study, we utilized PWR spectroscopy to analyze the biased signaling of the μ-opioid receptor (μ-OR) pre-bound with three distinct ligands: linear endomorphin, cyclic endomorphin, and lactomorphin. Interactions with three common intracellular transducers—β-Arrestin 1, β Arrestin 2, and Gi-protein—were investigated. Our findings revealed

ligand-specific biased agonism, demonstrating differential coupling of μ-OR-ligand complexes with each transducer. Conformational analyses conducted using the Schrödinger molecular modeling suite showed that linear and cyclic endomorphins share similar binding sites on the receptor. In contrast, lactomorphin engages a broader array of receptor residues, including both conserved and non-conserved amino acids. Notably, this broader interaction network appears to reverse the G-protein-biased signaling typically seen with μ-OR to a β-Arrestin-biased profile, potentially altering downstream signaling outcomes and therapeutic effects. Beyond opioid receptor research, our PWR spectroscopy method, when applied with supported lipid membranes, offers a promising platform for studying the molecular kinetics of beta-amyloid aggregation in an in vivo-like environment. This capability could be especially valuable for investigating Alzheimer's disease pathology across different brain subregions. In summary, PWR spectroscopy provides a powerful tool for elucidating the molecular mechanisms of biased agonism and can be instrumental in the development of next-generation, less addictive analgesics. Additionally, it holds promise for advancing our understanding of neurodegenerative processes, such as beta amyloid aggregation in Alzheimer's disease.

Jake Shaw – Biochemistry; Molecular & Cellular Biology #21

Research Faculty Advisor: Elisa Tomat, Chemistry and Biochemistry

Targeting Intracellular Iron in Cancer: Prochelation, Bioconjugation, and Localization Imaging Strategies

Iron is essential to the survival of cellular life, playing a critical role in growth and proliferation. Disruption of iron homeostasis results in failure of many systems across the human body. In anemia, low blood iron levels inhibit oxygen transport. Cancer cells display an augmented reliance on iron to fuel sustained proliferation rates and maintain a larger labile iron pool due to an upregulated iron uptake system. As a result, iron has been identified as a target for novel cancer therapeutics. Iron chelators have been clinically tested for cancer treatment but are often limited by non-specificity, resulting in systemic iron sequestration. Prochelation strategies seek to improve specificity by only releasing the active drug unit in the tumor microenvironment. The goal of this research is the development of novel strategies to target iron in the tumor microenvironment. Three approaches are employed to target intracellular iron.

First, a novel prodrug approach. Inspired by the design of antiviral medications, a phosphoramidate mask disguises iron chelators until taken up by a cell. Once in the cellular environment, enzymatic activation releases the toxic payload. The modular scaffold of this design provides space for a synergistic drug to be co-administered for increased therapeutic effect.

Second, a potential prodrug delivery system. A previous study revealed serendipitous conjugation between albumin and disulfide-masked iron prochelators. Molecular docking simulations validate prior results and elucidate suitable ligands for bioconjugation. Because cancer cells overexpress albumin receptors, conjugation to albumin may improve cellular uptake of iron chelators by tumors.

Third, an iron-dependent sensor for photoacoustic imaging. Imaging intracellular iron localization is of great interest, but existing compounds are limited. Pheophorbide-a is chosen for functionalization and several syntheses are performed. By using a pigment with a strong absorbance in the near-IR region, background overlap with heme may be avoided in imaging.

Sarah Shepherd – Biochemistry #51

Research Faculty Advisor: Jacob Schwartz, Pharmacology

How the Ewing Sarcoma fusion protein effects R-loops and mitochondria

The EWSR1 (Ewing Sarcoma Related Protein 1) gene can be translocated with transcription factor FLI1 to form EWS-FLI1, a fusion protein primarily responsible for Ewing Sarcoma tumors. EWSR1 is associated with regulation of RNA:DNA hybrids that displace a single stranded DNA (ssDNA), a structure called R-loops. Unscheduled R-loop formation may be linked with tumor formation. Cells expressing EWS-FLI1 have an increased number of R-loops

in comparison to cells lacking this protein fusion via removal of wild-type regulatory function of EWSR1. Increase in the global R-loop population causes destabilization of the nucleus and mitochondrial genome.

In this study, I investigate how the overexpression of EWS-FLI1 in HEK293 cells affects R-loops population, mitochondrial structure, and the subsequent mitochondrial functional disruption. Using immunofluorescence imaging, R-loop population and mitochondrial structure were observed. Immunofluorescence revealed an increase in global R-loop population and visual alteration of mitochondrial structure. Using MitoSOX mitochondrial activity assay, mitochondrial stress was observed. This data suggests that the overexpression of EWS-FLI1 causes an increase in global R-loop population and a visual alteration to mitochondrial structure, though overexpression on this level appears to lack the ability to produce a significant difference in mitochondrial dynamic activity.

Douglas Swango – Biochemistry; Pharmaceutical Sciences #39

Research Faculty Advisor: Pascale Charest, Molecular and Cellular Biology

Determining the Role of HRas Alterations on the PI3K/mTORC2/AKT Signaling Axis

Ras is a small GTPase mutated in approximately 30% of cancers, leading to hyperactivation, increased proliferation, and migration of tumors. Migration is of particular concern due to increased metastatic potential, which increases the risk of death from cancer. Ras has been associated with PI3K, AKT, and mTORC2, proteins involved in signaling pathways related to migration. However, its exact role in the PI3K/mTORC2/AKT signaling axis requires further study. I examined the role of Ras transformations and signaling perturbations on the axis by genetically altering HRas in MCF10A breast epithelial cells via overexpression or constitutively active mutation, EGF stimulation assays, pharmacological PI3K inhibition via wortmannin, and Ras knockdowns using siRNA. I found that both Ras-overexpressing and constitutively active cells saw increases in PI3K and mTORC2-dependent AKT phosphorylation compared to control cells upon EGF stimulation. PI3K inhibition, both in EGF stimulation assays and growing cells, led to inhibition of both PI3K-dependent and mTORC2-dependent AKT phosphorylation across all three cell lines, with more PI3K inhibitor required to produce a response in HRas-altered cells. PI3K inhibition also led to a reduction in Ras activity, only in control cells. Utilizing siRNA to knockdown Ras, I identified that reductions in Ras expression led to decreases in Ras activity, as expected, as well as subtle decreases in PI3K and mTORC2 activity. These results suggest that PI3K is critical to activation of both growth and migration signaling, although further investigation is warranted to determine mTORC2 localization and a specific role of GTPases such as Ras in this cell model.

Lauren Thaller – Biochemistry #2

Research Faculty Advisor: Michael Brown, Chemistry and Biochemistry

Insights Into Retinal Chromophore Dynamics During Rhodopsin Activation

Rhodopsin is a photoreceptive G-protein-coupled receptor (GPCR) located in the retina of the eye responsible for vision in dim light. Its activation involves retinal, the covalently bound chromophore, undergoing isomerization, which triggers a conformational change from the dark-state (11-cis retinal) to the equilibrium between the intermediate inactive Metarhodopsin-I (Meta-I) state (all-trans retinal) to the fully active Metarhodopsin-II state (all-trans retinal). This process is accompanied by a proton transfer from the protonated Schiff base of retinal to GLU-181, with water molecules playing a key role in stabilization. The purpose of this research is to develop accurate computational models of rhodopsin, focusing on retinal and its surrounding environment. By exploring the dark-state, Meta-I state, and both flipped and unflipped retinal orientations of the Meta-II state along with the varying water molecule distributions, we aim to create models that can predict the probabilities of these states and their contributions to activation. These models have implications for understanding GPCR mechanisms and informing drug design. Using tools like Pymol, Gaussview, Molden, and VMD, we simulate retinal, its surrounding amino acids, and structural waters and perform time-independent and time-dependent density functional theory (TIDFT and TDDFT) calculations. The results from these calculations provide the corresponding UV-visible spectra and the energy levels of the highest occupied molecular orbital (HOMO) and the lowest

unoccupied molecular orbital (LUMO). The focus is on building robust models that align with experimental findings, such as wavelength peaks for dark-state (~498 nm), Meta 1 (~380 nm) and Meta 2 (~478 nm), and refining our understanding of molecular interactions under different conditions. This work provides a foundation for creating predictive models that give us an insight into rhodopsin's activation mechanism and support broader GPCR research.

Samantha Thomas – Biochemistry #56

Research Faculty Advisor: Andreia Zago Chignalia, Anesthesiology

Gene Expression of Sepsis Induced Lung Edema in Syndecan-1 Knockout Mice

Understanding the lung edema pathophysiology requires a further understanding of the expression levels of genes responsible for maintaining lung fluid homeostasis. Current research in the Chignalia lab has shown that a Syndecan-1 knockout mice model is protected from pulmonary edema when they are subjected to a model of sepsis-induced lung edema. This mice model can be used to further investigate the pathophysiology of lung edema by exploring the varying levels of different gene expressions via qPCR analysis. The major gene expression levels that will be investigated include the cystic fibrosis transmembrane conductance regulator (CFTR), aquaporin 5 (AQP5), epithelial sodium channel (ENaC) and surfactant protein A, B, C, and D. (Add key results/conclusions, still running qPCR reactions).

Madeleine Tibayan – Chemistry #23

Research Faculty Advisor: Jeanne Pemberton, Chemistry and Biochemistry

Arginine-based Glyonic Liquids from Monorhamnolipids: Sustainable Synthesis, Characterization, and Potential Applications

Ionic liquids (ILs) are salts with melting points below 100 °C, known for their tunable physicochemical properties and potential applications in green chemistry. When combined with rhamnolipids derived from Pseudomonas aeruginosa, a novel subclass of ILs, termed glyonic liquids (GLs), can be formed. This project aimed to synthesize and characterize a new GL using arginine as the cation, providing a renewable and less toxic alternative to conventional ILs. Structural confirmation was achieved through ^{1}H, ^{13}C, and DEPT NMR spectroscopy, which verified the expected molecular framework. To confirm the mechanism of GL formation as a Brønsted–Lowry acid–base reaction, pH-dependent NMR experiments were conducted on the individual acid and base components, demonstrating proton transfer consistent with ionic liquid formation. Additionally, comprehensive thermal and viscoelastic analyses were conducted to investigate the material's properties. Characterization techniques included density measurements, rheology, thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC). A key finding was the confirmation that the arginine-based GL (Arg GL) remains in the liquid state at room temperature. Previous studies have shown that GLs can facilitate gas physisorption, and arginine's three amine functionalities suggest potential for enhanced CO2 chemisorption. This work has also begun to optimize thin films compatible with gas sorption measurements using a quartz crystal microbalance. Future work will continue this project, focusing on subsequent CO2 sorption measurements quantified using the Sauerbrey equation.

Jaden Todd-Nelson – Biochemistry; Molecular & Cellular Biology #47

Research Faculty Advisor: Anita Koshy, Neurology

Characterizing the Role of Ctr2 in Toxoplasma gondii Bradyzoite Differentiation and Latent Persistence

Toxoplasma gondii (T. gondii) is an intracellular parasite that persistently infects the central nervous system (CNS) of up to a third of the world's population. This CNS persistence can have significant consequences for the immunocompromised. To establish a persistent infection, T. gondii differentiates from its acute/lytic, fast-growing tachyzoite stage to a latent, slow-growing bradyzoite stage which persists within cysts. This project

focuses on a copper transporter, Ctr2, that is predicted to be dispensable in tachyzoites and shows higher expression in bradyzoite-inducing conditions. To determine the role of Ctr2 in stage conversion and persistence, we generated a T. gondii strain that lacks Ctr2 (IIΔctr2) as well as an ectopically expressed, HA tagged complement strain (IIΔctr2::Ctr2). As expected, IIΔctr2 parasites show no lytic cycle defects in vitro. However, under high pH, low CO 2 conditions, IIctr2 cysts show high levels of abnormal morphology, relative to wild-type and complemented strains. Consistent with a lack of copper driving this phenotype, copper supplementation rescues the IIΔctr2 cyst defect. In an in vivo infection model, at three weeks post-infection (wpi), IIΔctr2 parasites show a decreased CNS parasite burden compared to the parental/wild type strain. This finding suggests that our initially-hypothesized bradyzoite-specific defect could extend to the acute stage in vivo. Future studies will address the viability of bradyzoites within abnormal cysts in vitro; changes in Ctr2 localization under stress and copper-supplemented conditions; and characterization of a potential IIΔctr2 acute defect both in vitro as well as in vivo.

Monique Trujillo – Biochemistry #50

Research Faculty Advisor: Nam Lee, Pharmacology

ATAT1 Hyperactivation in Pancreatic Cancer Drives Tumor Growth by Enhancing DNMT1-dependent Silencing of NDRG4 Expression

Microtubule acetylation is closely linked to tumor progression and chemoresistance in many cancers. As the sole mediator of MT acetylation in vivo, alpha-tubulin acetyltransferase 1 (ATAT1) represents a key therapeutic target of metastatic growth. However, ATAT1 is detected at weak to moderate levels in most normal and cancerous tissues, thus hampering efforts to determine which tumor types are strong candidates for targeted inhibition. We previously reported that ATAT1 is activated upon phosphorylation at Ser237 by TAK1, a versatile kinase induced by TGF-b, BMPs, and various inflammatory cytokines. Here we employed the first of-its-kind phospho-Ser237 screening across multiple different human cancer cell lines and found that ATAT1 is distinctly hyperactivated in pancreatic ductal adenocarcinoma (PDAC). In mice, orthotopically transplanted PDAC cells harboring a Ser237-to-Ala knock-in point mutation showed impeded tumor growth relative to control. Based on quantitative proteomics, we determined that ATAT1 hyperactivation drives PDAC tumor growth by silencing NDRG4 expression through the AKT/GSK3-b pathway which we find upregulates DNMT1 protein translation. Collectively, these results support a fundamental role for ATAT1 in PDAC progression and downstream tumor modulators including NDRG4 as therapeutic targets.

Makaela Valencia – Biochemistry #44

Research Faculty Advisor: Paul Carini, Environmental Science

Cultivating the Unculturable: Integrated Statistical Design and Amplicon Sequencing for Isolating and Identification of Microorganisms from a Dryland Xeriscape Soil

Soil microbial communities play a crucial role in the sustainability and health of the planet's ecosystems, serving as invisible architects of soil health and ecological stability. Despite their ecological significance, our understanding of these communities remains incomplete, impeded by our limited ability to culture and characterize their individual microbes. This study aimed to address this key knowledge gap by employing a Taguchi-based experimental design combined with amplicon sequencing to systematically isolate and identify previously uncultured microbes from a dryland xeriscape soil. We used a multilevel (L27) Taguchi array, which tests multiple factors using just 27 experiments. This allows for a large experimental space to be covered via a minimum number of experimental runs, i.e., we simultaneously optimized multiple cultivation factors including pH, temperature, salinity, carbon concentration, oxygen conditions, light conditions, incubation times, electron acceptors, vitamin concentrations, carbon source, and media solidification type. Our results revealed that incubation time significantly affected species richness, with longer periods leading to reduced microbial diversity. While some cultivation factors showed trends in their effects on microbial communities, most did not reach statistical significance at p<0.05. This statistical approach allowed for the efficient optimization of multiple cultivation factors with fewer experimental steps, providing new insights into the hidden microbes of soil

microbial communities and establishing a foundation for future research on the taxonomic and functional characterization of previously uncultured microbes in dryland ecosystems.

Haley Vanhof – Biochemistry #37

Research Faculty Advisor: Marielle Hegetschweiler, Chemistry and Biochemistry

Examining the ATP Dependent Oligomeric State of Human Hsp60

Chaperonins play a crucial role in maintaining homeostasis in biological systems. Heat shock protein 60 (Hsp60) is a mitochondrial chaperonin that assists in protein folding and prevents protein aggregation. Additionally, it is implicated in neurodegenerative diseases such as Alzheimer's Disease. Hsp60's oligomeric states- monomeric, heptameric, and tetradecameric- experience conformational changes throughout its folding cycle, specifically after ATP binding. This study explores the molecular reconfiguration of cytosolic Hsp60 in real time utilizing charge detection mass spectrometry (CD-MS) and mass photometry. For these studies, the purification protocol was optimized to yield a low-salt sample, as required for mass spectrometry. Hsp60 was expressed in E. coli, purified with a novel low-salt protocol using immobilized metal affinity chromatography (IMAC) and size exclusion chromatography. The purity and assembly state were confirmed by Sodium Dodecyl Sulfate (SDS)- and Blue Native (BN)- Polyacrylamide Gel Electrophoresis (PAGE). To our surprise, CD-MS results revealed that Hsp60 monomerizes upon ATP binding. Additionally, we used mass photometry to validate the data. However, due to the low concentrations required for that measurement, the data could not initially be obtained. To overcome this, we built upon a surface PEGylation and amination protocol for mass photometry. The measurement now works well, and high concentrations of Hsp60 can be acquired—though the exact concentration limit is still being determined. This aspect of the project is still a work in progress.

Daniela Villacorta – Biochemistry #29

Research Faculty Advisor: Tarjani Thaker, Chemistry and Biochemistry

The role of ADCK4 in remodeling mitochondrial networks in kidney disease

COQ8B is a gene in higher-order organisms that encodes the mitochondrial protein known as ADCK4. This protein plays a role in a biosynthetic pathway that supports the formation of the molecule Coenzyme Q (Ubiquinone) in the body. Its function is critical for cellular homeostasis, especially energy production in the mitochondria, and in kidney disease. The goal of this project was to identify the effect of recently discovered mutations in ADCK4 found in patients with renal failure on mitochondrial homeostasis and energy production. We used immunofluorescent imaging microscopy to define morphological changes in the mitochondrial network of a model kidney cell line, COS-7 cells, caused by disease ADCK4 variants. Cells were transfected with wild-type, I346S, W520X, or a double mutant containing both I346S/W520X in ADCK4 and labeled with antibodies against ADCK4 and the mitochondrial protein, TOM20. The results of this work revealed distinct effects of each variant on driving mitochondrial aggregation or fragmentation compared to wild-type ADCK4, suggesting the role of this protein in not just ATP production, but also in regulating mitochondrial quality control mechanisms. Future directions will include determining which mechanism is driving mitochondrial aggregation and/or fragmentation and whether these effects are pathway-specific or the result of ATP depletion. The results of this work will allow us to better understand ADCK4 functions in kidney disease.

Dylan Weaver – Biochemistry #15

Research Faculty Advisor: Michael Taylor, Chemistry and Biochemistry

Nona-fluoro Quinolinium Cation as a Radical Photocage for Post-Translational Modification

Described here, the synthesis of nonafluorinated quinolinium sulfonate ester then its utilization for post-translational modification of biomolecules via photodecaging. Upon absorption of visible light, the sulfonate ester is decaged via photolysis creating an electrophilic C-centered nonafluoro radical that is trapped by π-nucleophiles

in biomolecules. Further experiments with this nonafluorinated quinolinium sulfonate ester involve post-translational modification of nanobodies and the study of protein-protein interactions with further implications in drug discovery.

Jamison White – Biochemistry #17

Research Faculty Advisor: Michael Taylor, Chemistry and Biochemistry

Light mediated chemistries for assessing interactions between biological and small molecule matter

Photochemistry offers a powerful approach for achieving precise and accurate protein modifications. In this study we report the synthesis of a thalidomide-based photoreactive molecule that incorporates a light-sensitive N–N bond and an alkyne handle for subsequent click chemistry. When irradiated with a defined wavelength, the N–N bond cleaves generating a nucleophilic nitrogen site for targeted covalent modification of proteins. Characterization by NMR spectroscopy, mass spectrometry, UV/Vis spectrometry and gel electrophoresis confirm the specificity of the light-mediated reaction, highlighting its potential for applications in chemical biology such as new functional protein discovery for drug targeting.

Bryce Wilson – Molecular and Cellular Biology; Biochemistry #40

Research Faculty Advisor: Andrew Paek, Molecular and Cellular Biology

Hydroxyl Radicals and Calcium Signaling As Central Regulators In The Cellular Response To Hydrogen Peroxide

Hydrogen peroxide (H2O2) and calcium (Ca2+) are fundamental signaling molecules that regulate many cellular processes and have been implicated in numerous diseases including cancer and neurodegenerative disorders. Understanding the crosstalk between these molecules in the context of oxidative stress is vital to our understanding of health and disease. Here, we partially elucidate the role of Ca2+ signaling in the activation of transcription factors during H2O2-induced stress. Through the inhibition of Ca2+ signaling in several manners, our results provide evidence that NFAT1, FOXO1, and p53 activation is Ca2+ dependent. We demonstrate that H2O2 activates NFAT1 through IP3 mediated Ca2+ release in a calcineurin independent manner. Additionally, AKT inactivation was found to be insufficient to drive Ca2+-dependent FOXO1 activation during H2O2 stress. Damage caused by hydroxyl radicals produced from the Fenton reaction between H2O2 and bivalent transition metal ions like Fe2+ and Cu2+ appears to be central to this response. These results suggest complex Ca2+-mediated TF activation pathways are used in the cellular response to oxidative stress potentially mediated through hydroxyl radical production.

Grady Zucco – Biochemistry #34

Research Faculty Advisor: Thomas Tomasiak, Chemistry and Biochemistry

Predicting drug interactions with monocarboxylate transporters expressed in cancer cells using AI generative structures and computational analysis

One of the defining traits of cancer cells and tumors is the aggressive rate of metabolism. In many cancer types, Monocarboxylate Transporter (MCT) proteins MCT1 and MCT4 facilitate this hypermetabolic state by cycling lactate in and out of the cell, which is used as a primary fuel source. Both MCT1/4 are overexpressed in multiple types of cancers including: soft tissue cancers, breast, brain, glioblastoma, cervical carcinoma, prostate, lung, gastric, renal, head and neck, colorectal, ovary, and adrenocortical cancer. Therefore, finding an inhibitor of both MCT1 and MCT4 is the focus of progressive cancer treatments. There is currently a clinical trial for an MCT1-specific inhibitor (AZD3965), but current MCT4-specific inhibitors are limited as there is a lack of structural data of MCT4. By using AI generative structures and computational analysis, I investigated the interfaces between MCT1/4 and various inhibitors to produce a better understanding of potential drug design for future research. From the structures and multiple sequence alignments, I found the key residues inside of the binding pocket of MCT4 which can be used as the focal point of drug design as an inhibitor of MCT4, as well as a potential dual

inhibitor of both MCT1 and MCT4. These findings have the potential to lead to novel cancer treatments, but the methodology can also be applied to a wide range of proteins that do not have a Cryo-EM structure yet.

RESEARCH PRESENTATIONS

Daniel Aranda – Chemistry #83

Research Faculty Advisor: Thomas Gianetti, Chemistry and Biochemistry

Synthesizing New Linkers For Carbenium to Pyrene Photon Upconversion

Photon upconversion is the phenomenon of absorbing two lower energy photons and emitting one higher energy photon. This process can be applied to many different areas including solar energy harvesting, bioimaging, catalysis, and others. A molecule synthesized in our lab pyrene-ADOTA, which consists of a tri-aryl carbocation core functionalized with pyrene, has shown to be capable of upconversion. Through modification of the linker between the carbocation core and pyrene, improvement on the upconversion efficiency may be achieved. As well as open the door to upconversion of lower wavelengths of light.

Cole Bellomo – Chemistry #88

Research Faculty Advisor: Jeffrey Pyun, Chemistry and Biochemistry

Fabrication and Mechanical Testing of Inverse Vulcanized Sulfur Polymers

Conventional methods for producing IR lenses involve the use of super-critical and expensive materials, such as germanium. Due to the high cost and demand of these lenses, especially for defense and automotive industries, the Pyun group has proposed an alternative method that involves the copolymerization of sulfur with an organic monomer, dubbed inverse vulcanization. Initial research showed that the mechanical properties of the sulfur-polymer were lacking. Unreacted sulfur remained in the lens, which affected the mechanical and thermal properties. To improve these properties, the Pyun group developed varying conditions when fabricating these polymers. The conditions included varying the weight composition of the co-polymers, the temperature of the polymerization, and the thermal curing temperatures after polymerization. For this research project, various fabrication techniques were employed to improve the mechanical properties of the polymers. Based on initial observation, increasing the composition of sulfur improved the thermal imaging of lenses and improved the mechanical properties of the polymer.

Anna Campbell – Biochemistry #78

Research Faculty Advisor: Matthew Cordes, Chemistry and Biochemistry

Venom recruitment of a phospholipase in recluse spiders included an activity-enhancing truncation

Venom components are often recruited from pre-existing proteins encoded in the host's genome. Such recruitment events can be associated with modifications that may increase the protein's suitability towards its new role. In the case of a phospholipase D family found in numerous invertebrates, venom functionalization by recluse spiders correlates with the loss of a 30-40 residue C-terminal tail. The role of the C-terminal tail was investigated via insertion of a stop codon to truncate the tail in a non-venom enzyme from the recluse spider Loxosceles rufescens. Preliminary activity comparisons with the wild type show a sphingomyelinase activity gain of one to two orders of magnitude in the tailless mutant. Examination of Alpha Fold structures suggests the tail may bind in a potential allosteric site, which could be the basis for the observed inhibition. The tail’s terminal cysteine could also be involved in disulfide bridge exchange, as the difference in SDS-PAGE mobility between the oxidized forms versus corresponding reduced forms of the non-venom enzymes suggests disulfide isomerism. We speculate that the C-terminal tail may perform a regulatory function, as arachnids also possess sphingolipid

substrates in their membranes, yet presumably avoid uncontrolled toxic enzymatic activity in endogenous use. In the context of venom function, however, deregulation of catalytic activity is potentially beneficial.

Kylie Ernst – Biochemistry #72

Research Faculty Advisor: Andrew Paek, Molecular and Cellular Biology

Transcription Factor Activation During Decidualization of Endometrial Stromal Cells

The process of decidualization involves the differentiation of endometrial stromal cells into a structure called the decidua. This occurs in menstruating species in preparation for embryo implantation and pregnancy. In a normal menstrual cycle, fluctuating levels of progesterone influence the expression of genes that drive decidualization. Progesterone is withdrawn in the absence of embryo implantation, signaling for cell death and ultimately the shedding of the decidua through menstruation. This hormone cycle was modelled in human endometrial stromal cells (HESCs) to identify and elucidate patterns in the activation of transcription factors, such as FOXO1 and p53, that regulate this process. These results could provide insight into the causes of endometriosis and other aspects of women's health.

Luke Fasse – Chemistry #82

Research Faculty Advisor: Thomas Gianetti, Chemistry and Biochemistry

Metal Coordination to Organic Photocatalyst for Bifunctional Metallaphotoredox Catalysis

Recently, dual catalysis using a combination of photocatalysts and transition metal catalysts has emerged as a transformative strategy in organic synthesis, enabling cross-coupling reactions with unprecedented efficiency and selectivity. In the presence of light, these systems have been shown to facilitate the regeneration of metal oxidation states necessary to initiate and maintain catalytic cycles. Among these approaches, bifunctional photocatalysts capable of coordinating transition metals have gained significant attention for their potential to support synergistic reactivity through spatial proximity. The propinquity of catalytic centers expediates electron transfer events, better conjoining the two catalytic cycles. Despite this potential, bifunctional photocatalysts that combine metal coordination and photocatalytic activity within a single molecule remain an underdeveloped area of research. A promising candidate for such systems is organic photocatalyst dimethoxyquinacridinium (DMQA+) and its related derivatives. Herein, I report the synthesis of a bifunctional DMQA+ via constructing a β-diketiminate (nac-nac) ligand motif within the DMQA+ scaffold.

Elizabeth Ghartey – Biochemistry; Math; French #65

Research Faculty Advisor: Emmanuel Katsanis, College of Medicine

Characterizing a patient-derived neuroblastoma cell line as an immunotherapeutic model

Neuroblastoma is a pediatric cancer of the sympathetic neuronal tissue. Its clinical and biological attributes vary significantly, which often complicates effective treatments. In this study, we phenotypically characterized a patient-derived neuroblastoma cell line (M418). After culturing an M418 line that was virally infected with a luciferase reporter (M418-luc), we used the puromycin resistance gene to select for stable integration, and determined that the luciferase signal was stable. In our preliminary in vivo tests, intravenous delivery of M418-luc cells caused rapid tumor metastasis and vast tissue distribution. We opted for orthotopic retroperitoneal injections to achieve a more localized tumor distribution. In preparation for future functional assays, we performed flow cytometry to profile the expression of immune ligands on tumor cells. The assays determined that the M418-luc cell line has an array of immune markers on its surface that could potentially be used in targeting these neuroblastoma cells. Based on the surface expression of immune ligands and our in vivo distribution, this cell line of neuroblastoma is a favorable model for tumor growth, and it will be beneficial for testing potential therapies against neuroblastoma.

Jaxon Goddard-Westland – Chemistry; Mathematics #87

Research Faculty Advisor: Oliver L.A. Monti, Chemistry & Biochemistry

2D Material Sample Transfer Station for Fast Fabrication of Van der Waals Heterostructures.

While there are various methods of fabricating Van der Waals (VdW) heterostructures, mechanically stacking layers is among the most efficient for creating small (50-500 μm scale) heterostructures ideal for electronic structure analysis experiments. With a standard optical microscope, few layer (2-10 layers) and even atomically thin monolayer crystals with high optical contrast on a given substrate (most commonly SiO_2 with 300 nm oxide layer) can be identified based on the unique color corresponding to the number of layers. Subsequently, crystals of interest can be "picked up" using layered temperature controlled viscoelastic polymer stamps. Once adhered to the polymer stamp crystals can be removed from the substrate. Once an initial crystal has adhered to the polymer, additional layers adhere to the initial crystal through interlayer VdW interactions. Once all the desired crystal layers have been picked up the polymer stamp can be heated, releasing the layer of polymer in contact with the heterostructure. In doing so, the heterostructure can be deposited with a high degree of precision onto the substrate. This method provides a way to create complex stacks of 2D materials demonstrating a wide array of unique inter- and intra-layer electronic properties.

Natasha Graham – Biomedical Sciences #93

Research Faculty Advisor: Jeffrey Pyun, Chemistry & Biochemistry

Sulfenyl Chloride Commodity Chemical Feedstocks for High Refractive-Index Photopolymers for Plastic Optics and 3D- Printing

This study presents a cost- effective approach to creating high refractive index (n ~ 1.57) of photopolymer resins in plastic optics, photopatterning, and advanced 3D printing applications. Our work introduces disulfide methacrylate resin (DSMR), synthesized by reacting allyl methacrylate with sulfur monochloride (S_2Cl_2), yielding a stable, free-radically polymerizable resin that achieves high transparency, low birefringence, and robust thermomechanical properties. DSMR is sulfur- based and thus is more economical and sustainable than traditional photopolymers. DSMR allows for the fabrication of bulk optical glass with thickness control from 1-30 mm and enables high-fidelity precision optics through rapid photopolymerization, minimizing fabrication times compared to conventional thermal curing methods. The DSMR resin's versatility is demonstrated across multiple processing techniques, including molding, diamond-turn machining, and digital light processing (DLP) 3D printing using High Area Rapid Printing (HARP) technology. The study validates the high RI and transparency of DSMR in both the visible and near-infrared spectrum. The adaptability of DSMR photopolymers across various fabrication methods highlights its value as a cost- effective means of producing a high refractive index photopolymer. Additionally, DSMR's low toxicity and compatibility ensure safe use for plastic optics in various consumer and industrial applications.

Wyatt Hendricks – Nursing #76

Research Faculty Advisor: Marielle Walti, Biochemistry

Nuclear Magnetic Resonance Exploration of Large Disease Relevant Proteins

Heat Shock Proteins (HSPs) are a class of proteins that maintain cellular homeostasis during periods of cellular stress such as changes in pH, changes in temperature, and oxidative stress. Two of the proteins in the heat shock class, HSP10 and HSP60 are particularly of interest because of their roles in many disease processes like cancer, neurogenerative disease, and cardiovascular disease. Understanding HSP10 and HSP60's role in these diseases begins with understanding how these proteins function normally. Here, we focus on HSP10's role in homeostatic cellular function. One way to investigate these interactions of HSP10 is through Nuclear Magnetic Resonance (NMR), where the atoms within the backbone of the proteins can be identified and assigned to specific amino acid residues within the backbone. Due to the size of HSP10, non-mobile regions (~85%) are not able to be

visualized through regular methods of NMR because of the nuclear shielding of the protons within the protein. To mitigate the effects of proton nuclear shielding we can replace the protons in the sample with deuterium, an isotope of hydrogen that is not visualized on NMR. Here we show the purification process and backbone assignment of deuterated, nitrogen, and carbon labeled HSP10 (DNC-HSP10). This assignment allows future research in how specific amino acid residues interact with native substrates, such as known native substrates, MDH1 and SOD.

Andrea Hernández – Biochemistry; Molecular & Cellular Biology #69

Research Faculty Advisor: Wei Wang, Pharmacology and Toxicology

Efficiency Assessment of BRD4/PLK1 Dual Degraders: A Promising Approach to Cancer Treatment

Small-molecule inhibitors block the enzymatic activity of crucial proteins involved in cancer proliferation. However, achieving high affinity often requires high drug concentrations, increasing the risk of toxicity, drug resistance, and undesired biological outcomes. Molecular glues are novel molecules that address these problems. They alter the surface of either an E3 ligase or a protein of interest to stabilize their interaction, catalyze the ubiquitination of a target protein, and promote further proteasome degradation. Nevertheless, most molecular glues against cancer focus on the degradation of a single protein, ignoring the possible potency increment and toxicity reduction when degrading a pair of tumor-related proteins. We attempted to evolve the mechanism of molecular glues by the simultaneous degradation of two overexpressed proteins in multiple cancers: BRD4, a transcriptional and oncogene expression regulator; and PLK1, a serine/threonine-protein kinase that regulates mitotic checkpoints. The degradation ability, apoptosis/DNA levels, and cell viability of dual degrader candidates in different cancer tumor cell lines were tested by Western Blot assay, flow cytometry, and CCK8 assay. The BPD-05 compound displayed great degradation and similar cell activity to the commercial BRD4/PLK1 inhibitor. In vivo studies demonstrated that treatment with the BPD-05 compound leads to significant tumor regression, inhibition of the chronic myeloid leukemia pathway and promotion of apoptosis.

Hanami Inagaki – Chemistry #97

Research Faculty Advisor: Adam Printz and Patrick Lohr, Chemical and Environmental Engineering

Revealing Solvent Coordination in Perovskite Precursors through a Combined Spectroscopy/TD-DFT Approach

Metal halide perovskites are promising materials for applications in photovoltaics due to their outstanding optoelectronic properties, such as high carrier lifetime, mobility, and power conversion efficiency. Understanding solvent and ligand coordination chemistry in these materials is critical for designing precursor solutions—"inks"—for the upscaled manufacturing of perovskite thin films for next-generation photovoltaic technologies. UV-Vis spectroscopy provides a quick, robust approach for identifying the presence of different metal-halide coordination states within perovskite ink formulations. By coupling these experiments with time-domain density functional theory (TD-DFT) calculations, we can gain valuable insight into the coordination states of perovskite precursors. In this work, we use this combined simulation/experiment approach to investigate the solution chemistry of mixed formamidinium/methylammonium lead iodide (FA0.4MA0.6PbI3) perovskite with different solvents. Our results indicate that the addition of DMSO shifts the coordination of iodoplumbate complexes towards lower coordination numbers, resulting in increased absorbance for these species.

Pedro Juarez – Chemistry #94

Research Faculty Advisor: M. Leandro Heien, Chemistry and Biochemistry

Chiral Analysis of Ketamine and its Metabolites Using LC-MS/MS

The Heien group developed and validated a chiral LC-MS/MS method in human plasma for the separation and quantification of (R) and (S) enantiomers of ketamine and its metabolites—norketamine, hydroxynorketamine, and dehydronorketamine. Ketamine has shown therapeutic potential in reducing abnormal involuntary

movements (AIMs) associated with levodopa-induced dyskinesia (LID), a motor complication affecting most Parkinson's disease patients undergoing long-term L-3,4-dihydroxyphenylalanine (L-DOPA) therapy. Ketamine and its metabolites exhibit enantioselective pharmacokinetics, therefore accurately quantifying each chiral species is essential for the understanding of their individual contributions. Validation was performed for linearity, precision, accuracy, recovery, matrix effects, enantiomeric resolution, and stability. Preliminary results demonstrate enantiomeric resolution and reliable quantification of all analytes. Once validated, this method will serve for characterizing the enantioselective pharmacokinetics of ketamine and its metabolites, providing the fundamentals for future studies investigating roles in modulating dyskinesia in Parkinson's disease therapy.

Joseph Jung – Chemistry #91

Research Faculty Advisor: Jeffrey Pyun, Chemistry and Biochemistry

Characterization of IR Transparency of Inverse Vulcanized Sulfur Polymers

Plastic materials for infrared (IR) optics are extremely limited due to the absorbance that occurs from strong C-C and C-H molecular vibrations in the Long Wave IR (LWIR) region. New IR transparent polymeric materials were developed by polymerizing large weight percent (>50%)elemental sulfur (S8) with organic monomer to reduce minimize unwanted molecular vibrations. This polymerization method, termed Inverse vulcanization, uses sulfur as a feedstock, to replace the germanium application. Containing high sulfur content increases the percent transparency in LWIR region. This synthesis also allowed for a decrease in germanium mining and the fabrication and refurbishment of new IR optics with sulfur, reusing materials from sulfur mining.

In the previous study in Pyun group, it was observed and found that there is no standardized way to accurately measure the IR % transmission of different materials, composition, and thickness. Therefore, the first demonstration of comparing samples was done with a thermal IR imaging system that uses a hotplate as a photon source, PMMA IR imaging target, and replaces the Ge lens with a poly(S-r-NBD2) Fresnel lens and compared. The next method was using different modes of FTIR, MCT, and DTGS. The main difference in the detection modes is how the materials interact with the material, and it was observed that the MCT has better sensitivity and transparency.

Isaac Kailat – Biochemistry #86

Research Faculty Advisor: Michael Marty, Chemistry and Biochemistry

Fluorescent Nanodisc Reporters for Lipidomic Lipid Exchange Mass Spectrometry

Nanodiscs are an emergent platform for membrane-interfacing biophysical analysis and therapeutic delivery. Previously, membrane proteins lipid-binding affinity and thermodynamics were characterized through a lipid exchange mass spectrometry workflow. This project aims to develop specialized nanodisc reporters for multiplexed lipid exchange experiments. This project validated a family of chimeric apolipo-mimetics fused with fluorescent barrel proteins that self-assemble into nanodisc reporters. Green and red fluorescent membrane scaffold protein constructs were synthesized and assembled into nanodisc scaffolds. Stokes radii and native mass spectrometry data were used to characterize the reporters. Lastly, new reporters using the self-labelling HaloTag mechanism are being explored.

Nolan Knapp – Chemistry #92

Research Faculty Advisor: Jeffrey Pyun, Chemistry and Biochemistry

Optical Sample Fabrication Methods for High Sulfur Content Polymers

Each year, global petroleum refinement produces vast quantities of elemental sulfur, over 7 million tons in excess of global demand. To address this surplus, the Pyun group has developed several novel inverse-vulcanized polymers that incorporate elemental sulfur and organic monomer feedstocks to create inexpensive and optically

transparent materials. Among these materials is poly(S-r-(NBD)2), which exhibits both high long-wave infrared (LWIR) transparency and high refractive index, making it compatible with a variety of optical fabrication techniques. Through the utilization of fabrication methods such as solution casting and melt pressing, poly(S-r-(NBD)2) has been successfully processed into a variety of optical samples with variable shape, thickness, internal crosslinking, and surface morphology. The customizable nature of poly(S-r-(NBD)2) samples presents clear advantages over conventional germanium optics, which rely on expensive and highly specialized manufacturing processes.

Caleb Konecek – Biochemistry; Molecular & Cellular Biology; Spanish #79

Research Faculty Advisor: Pascale Charest, Molecular & Cellular Biology

Role of Rap1 versus PIP3 in the Regulation of mTORC2

Chemotaxis is a directed cell migration in response to external chemical stimuli that is critical for biological processes like development and immune response. Chemotaxis dysregulation has been linked to diseases spread like cancer metastasis, but its disruption isn't fully understood. Research has shown that the mechanistic Target of Rapamycin Complex 2 (mTORC2) is crucial for chemotaxis regulation and cytoskeleton structural protein rearrangement. Despite mTORC2's implications in chemotaxis, its precise role and regulation are unclear. Recently, we have identified the small GTPase Rap1 as a binding partner of the SIN1 component of mTORC2, and experiments have linked Rap1 overexpression to increased mTORC2 activity in mammalian cells. Additionally, evidence suggests that the membrane phospholipid PI(3,4,5) (PIP3) similarly regulates mTORC2 activity by binding it to the plasma membrane with experiments demonstrating decreased activity following inhibition of PIP3 production. Although Rap1 and PIP3 positively regulate mTORC2, there is a critical gap in understanding their relationship in its regulation and localization to the plasma membrane. Our research attempts to clarify their relationship as binding partners of SIN1 to provide insight into chemotaxis and disease processes. We hypothesize that Rap1 and PIP3 independently regulate mTORC2 activity in HEK293 cells by playing similar roles in its localization. To test this, we over expressed Rap1 and used a PIP3-production inhibitor, examining their effects on mTORC2 activity in HEK293 cells in response to stimulation by insulin, a strong activator of mTORC2. So far, our research shows no significant difference between the effects of Rap1 and PIP3 on mTORC2 activity.

Peter Luu – Biochemistry #67

Research Faculty Advisor: Elhelaly Waleed, Division of Cardiology

HMEL: Hypoxic Myocyte-enriched Essential for Life

Luu, P. 1* ., Ngo, N. 1* ., Elghamry, AS 2,3 ., Elhelaly, WM 2,3 .

College of Science, University of Arizona, Tucson, AZ Division of Cardiology, Department of Internal Medicine University of Arizona, Tucson, AZ. Sarver Heart Center, University of Arizona, Tucson, AZ.

It is now known that adult mouse and human hearts experience modest cardiomyocytes (CMs) turnover, which is mainly mediated by the proliferation of pre-existing CMs, even though the adult mammalian heart is incapable of meaningfully recovering functionally following substantial CMs loss. This turnover capacity is thought to be mediated by proliferative competency of a specialized cardiomyocyte population, rather than a stem cell. The maintenance and proper function of stem or progenitor cells in various organs depend on the hypoxia inducible factor 1 alpha (Hif-1α) subunit being stabilized, as they live in microenvironments that are relatively hypoxic. We recently identified a rare population of hypoxic/cycling CMs based on stabilization of the oxygen dependent domain (ODD) of Hif-1α. These CMs demonstrated clonal expansion and made a significant contribution to neo-cardiomyogenesis in the adult heart. Additionally, we used RNA-seq to conduct a differential transcriptomic analysis comparing hypoxic ODD CMs with normoxic CMs (1), in order to identify CMs turnover regulators and clarify the mechanism by which these ODD CMs are maintained in a hypoxic/cycling state. Conceivably, these genes may serve as therapeutic targets in order to stimulate adult CMs to proliferate and regenerate the heart.

Here we present data on a previously unidentified gene that we named "Hypoxic Myocyte-enriched Essential for Life" (HMEL). We found that HMEL was significantly upregulated by 9.3-fold in the ODD CMs compared to the normoxic ones (1). Despite having 95.8% homology with its human ortholog and being conserved across many species, the mouse gene HMEL's protein sequence showed no homology to any other known genes, according to phylogenetic and homology analysis. While this might indicate HMEL has a distinctive function, it also signifies a challenge to elucidate its function.

Preliminary results indicate that HMEL protein expression in the heart follows the transition from the hypoxic/proliferative neonatal heart to the normoxic/non-proliferative adult heart in a descending manner. To better understand HMEL function in vivo, we created a genetic loss of function mouse model. By mating heterozygous knockout mice, we failed to obtain any homozygous pups at birth by genotyping. In addition, the litter size was smaller than expected with an average of 4.5 pups per litter compared to an average of 8 pups per litter when their wild-type (WT) littermates were mated. This suggests that HMEL is a recessive embryonically lethal allele. To determine the approximate timing of embryonic lethality, we created multiple timed crosses between the heterozygous littermates, then collected uteri and dissected conceptuses at different gestational ages starting at E10.5. At E10.5, we could see smaller conceptuses resorbed in the uterus with gross anatomy and LacZ staining, further confirming embryonic lethality. When these conceptuses were dissected, they lacked both yolk sac and embryonic tissue. At E8.5, we were able to find and collect tissue samples from the highly resorbed homozygous knockout embryos using H&E sections, laser dissection and subsequent genotyping, further confirming the recessive embryonic lethality phenotype. In addition, we collected decidua at E6.5 for H&E staining, as well as Ph3 and TUNEL immunofluorescence to further elucidate the lethality phenotype. Our results showed significant anatomically deformed homozygous knockout embryos compared to the heterozygous and WT embryos, a significant decrease Ph3 and an increase in TUNEL staining in homozygous knockout compared to both heterozygous knockout and WT, which in turn showed no significant difference whether histologically or by Ph3 or TUNEL staining. To further characterize homozygous knockout embryos and deduce the precise timing of lethality, we collected and cultured blastocysts from heterozygous knockout crosses at E4.5. We then distinguished homozygous knockouts from heterozygous knockouts and WT by individually genotyping each blastocyst after dark-field microscopic imaging. Our results confirmed that homozygous knockout blastocysts are still viable at this gestational age and have the same histological features as heterozygous knockout and WT embryos. To further investigate the role of HMEL in embryonic stem cells (ESCs), we cultured ESCs from blastocysts from a heterozygous knockout cross. However, only ESCs from heterozygous knockout and WT blastocysts survived, while ESCs from homozygous knockout blastocysts did not. We conclude that HMEL is a recessive embryonically lethal allele where its haploinsufficiency is tolerated and that the time of embryonic lethality is between E4.5 and E6.5, i.e., peri-implantation lethality. We also hypothesize that HMEL is essential for the viability of the ESC, especially during the in vivo transition of the preimplantation embryo from ~8.7% oxygen in the oviduct decreasing to a more hypoxic 1.5–2% oxygen in the uterus (2).

HEML has been briefly mentioned in some publications as being involved in splicing (3). To elucidate the putative protein partners of HMEL, we generated two HEK239 cell lines in which we overexpressed HMEL human sequence flag-tagged at the N- and C-termini by lentiviral transduction. We then performed immunoprecipitation (IP) with Flag AB and confirmed overexpression by western blotting of both IP and IgG samples with both Flag and HMEL-specific AB. Our results confirmed the presence of HMEL in both input and IP samples, but not in the IgG sample. We then sent the IP and IgG samples for MASPEC analysis. MASPEC results showed that HMEL was one of the most abundant proteins in the IP sample, further confirming successful transduction. More importantly, the GO analysis of the pulled down proteins using David's analysis tool revealed an enrichment of biological processes involved in the regulation of RNA binding, RNA processing, mRNA splicing and RNA splicing. Thus, we hypothesize that HMEL may be a novel RNA-binding protein. We used the Antibody IP Validation Kit (Eclipsebio) to confirm the RNA binding ability of HMEL. Briefly, HEK293 cells overexpressing either N-terminally or C-terminally FLAG-tagged HMEL protein were grown to approximately 70% confluence and UV-cross-linked at 400 mJ/cm2. After lysis, cell lysate corresponding to approximately 50 µg of RNA was subjected to partial RNase I fragmentation and immunoprecipitation using α-FLAG magnetic beads. The washed bead fraction was end-repaired and ligated to biotinylated RNA oligo. Biotinylated RNA was separated by 4–12% NuPAGE and detected with streptavidin-HRP. Both N-terminal and C-terminal FLAG-tagged proteins were equally able to bind to RNA.

Further experiments will include identifying HMEL binding sites in the transcriptome using eCLIP. In addition, since the time of lethality coincides with the gastrulation process (E6.5), we decided to test the effect of HMEL gain and loss of function on the ESC differentiation process. However, because ESC culture from the homozygous knockout blastocysts is not possible, we decided to isolate and culture primary fibroblasts from F/F and WT mice, then reprogram them into iPSC (using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit) while knocking out and overexpressing HMEL.

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Elise MacKirdy – Chemistry #90

Research Faculty Advisor: Jeffrey Pyun, Chemistry and Biochemistry

Processing and Fabrication of Sulfur-based Copolymers

In 2013, the Pyun Group published the first demonstration of a new polymerization method using sulfur with organic comonomer, termed Inverse Vulcanization (IV). These polymers have desirable properties due to the high sulfur content (> 50 wt%) such as increased Infrared transparency, increased refractive index, and high processability. Depending on the organic comonomer the resulting polymer requires development of new fabrication parameters and procedures than non-sulfur-based thermosets and thermoplastics. This poster discusses current techniques for Sulfur polymer processing, challenges in monomer choice, and application driven sample creation.

Tyler Martinez – Chemistry #85

Research Faculty Advisor: Katrina Miranda, Chemistry and Biochemistry

Using Nanodiscs to Study Lipid Exchange

Lipids are known to modulate membrane protein structures and functions. To study lipid binding affinity to different membrane proteins, nanodiscs, a membrane mimetic, can undergo lipid exchange (LX). Nanodiscs used to study lipid exchange are assembled with lipids, membrane scaffold protein (MSP), membrane proteins, and detergent. After growing MSP from BL21 E. coli, immobilized metal affinity chromatography (IMAC) is used to purify Histidine (His) tagged MSP. The additional step of adding Tobacco Etch Virus protease cleaves the His-tags, and the His-cleaved MSP is purified again through IMAC. This allows the presence or absence of His tags from nanodiscs, enabling post- LX purification through IMAC. To separate different nanodisc populations pre-LX size exclusion chromatography is used. We established an LX control by observing the LX between nanodiscs without membrane proteins. We have successfully exchanged 100% phosphatidylcholine (POPC) nanodiscs with 90% POPC and 10% cholesterol nanodiscs to see lipids reach an equilibrium of ~95% POPC and ~5% cholesterol in both populations of nanodiscs. Once an exchange is performed with a membrane protein nanodisc, this equilibrium of lipids will shift, indicating a binding affinity for certain lipids to that protein. LX is analyzed using liquid chromatography coupled with mass spectrometry (LC-MS). Here, we are interested in how cholesterol impacts the structure and function of serotonin receptors. Recent studies show that patients who are taking cholesterol lowering medications experience increases in negative mental health effects, but it is unclear what this interaction looks like in natural systems. Future directions include using natural brain polar lipid extract within this process, and inserting the M2 influenza membrane protein, which has known interactions with cholesterol, into a nanodisc to observe its exchange behavior. Furthermore, we will observe the kinetics of cholesterol

exchange between empty nanodiscs to gain insight into the rate at which cholesterol exchanges between nanodiscs.

Gwendolyn McKay – Chemistry #71

Research Faculty Advisor: Catharine Smith, Pharmacology and Toxicology

Lysine Deacetylase-Containing Complexes Aid in Facilitating Transcription of Glucocorticoid Receptor-Targeted Genes

It has been widely accepted that lysine deacetylases (KDACs) facilitate the repression of gene transcription through their post-translational modification of histone proteins. However, recent research has revealed that KDAC1, a Class I lysine deacetylase, is able to activate glucocorticoid receptor (GR) target gene transcription. Many factors are unknown about the function of KDAC1 as an activator of GR transcription, therefore we are currently focusing on determining how the KDAC1-containing protein complexes are important for GR transcription, and how they can be located within the genome. There are various kinds of protein complexes which contain KDAC1, but the Corepressor of Repressor Element-1 Silencing Transcription factor (RCOR/CoREST) complex has been shown to be the most significant promoter for transcription of GR-regulated genes. In the RCOR/CoREST complex, there exists a scaffold protein of which one variation–RCOR3–has been observed to play a crucial role in KDAC1 function. Through a process of depleting RCOR3 protein from the RCOR/CoREST complex, we have observed impairments in basal and induced transcription of four GR targeted genes, indicating the scaffold's necessity to KDAC1 function. Moving forward with the knowledge of RCOR3's importance, we are developing a plasmid with a biotin-ligase insert in order to observe–through proximity profiling–other proteins that may interact with the complex. By identifying which transcription-necessary proteins are biotinylated, we can then identify those which are acetylated, and examine their function as possible substrates for KDACs. Researching Class I KDACs and their substrates can provide a deeper understanding of their inhibitors, which can aid in our understanding of how these drugs affect endocrine pathways.

Ethan McNew – Molecular & Cellular Biology; Biochemistry #66

Research Faculty Advisor: Geoffrey Gurtner and Dr. Kellen Chen, Department of Surgery

Histological analysis of severe foreign body response induced by wireless mechanical stimulation

Foreign body response (FBR) is a state of sustained, chronic inflammation in response to implanted biomedical devices. FBR leads to fibrotic encapsulation of a biomedical device, which reduces device biocompatibility and is the source of >90% of biomedical device failures in humans [1].

Based on our previous research [2], we developed a novel, wireless mechanically stimulating implant (MSI) to increase extrinsic tissue mechanical forces and promote human-like FBR in a murine model. Mice were assigned to either no-stimulation (NS, control) or MSI groups, and corresponding devices were implanted. FBR capsules from 82 mice were collected at postoperative day (POD) 10 and POD 28. We present a histological analysis of the resulting fibrotic capsules, collecting average thickness to verify the efficacy of our model in replicating human-like FBR.

MSI caused significantly thicker FBR capsules. At POD 10, mechanical stimulation caused capsule thickness to increase 44.19% between NS and MSI groups, from 176.59 um to 254.62 um ($p = 0.01$). At POD28, mechanical stimulation caused capsule thickness to increase 46.48% between NS and MSI groups, from 263.03 um to 385.28 um ($p = 0.03$). MSI in murine models successfully replicate human-like FBR by driving capsule formation around the implanted device, reaching capsule thicknesses comparable to reported human levels (~351.4 um) [3]. These findings provide a foundation for developing targeted therapeutics to mitigate implant-related FBR and reduce implant failure in biomedical devices.

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Akshay Menghani – Biochemistry; Mathematics #75

Research Faculty Advisor: Katherine Rhodes, Immunobiology

Characterization of Commensal Neisseria Polysaccharide Capsule

The genus Neisseria consists of a diverse set of gram-negative bacteria which exhibit both pathogenic and commensal behavior in their hosts. Human commensal Neisseria are common members of the mucosal microbiota, colonizing the oral cavity and nasopharyngeal regions. These species have high genetic similarity with the pathogens N. gonorrhoeae and N. meningitidis and encode several shared host-interaction factors. By studying host-interaction factors within commensal Neisseria, we aim to better understand the biological mechanisms of noninfectious colonization. This study focuses on the polysaccharide capsule—a layer of complex sugar molecules surrounding the bacterium's outer membrane. We utilized molecular cloning and gene expression techniques to examine capsule biosynthesis in N. subflava, a commensal of the human upper respiratory tract, and N. musculi, a commensal of the mouse oral cavity and gut. We deleted biosynthesis genes in the capsule locus of N. subflava and N. musculi and tested the mutants for capsule production using SDS-PAGE and Alcian blue staining. Both species were found to encode for a capsule phenotype, while N. subflava was found to have increased capsular expression at higher temperatures. Future directions include further characterizing gene expression under varying environmental conditions to determine how they are regulated. Functional assays will also be performed to better understand the role of capsule in commensal colonization and survival. Our findings will contribute to the understanding of the basic mechanisms of Neisseria adaptation and host interaction.

Jacob Narr – Molecular & Cellular Biology #89

Research Faculty Advisor: Jeffrey Pyun, Chemistry and Biochemistry

Disulfide Glass: A New High RI Polymer for Precision Optics and Photonics

We report the development of a novel negative photoresist from commodity monomers utilizing sulfenyl chloride inverse vulcanization (SC-IV) and thiol-ene post polymerization to afford a low-cost alternative to commercial photoresists. SC-IV combines sulfur monochloride (S2Cl2), a chemical derived from elemental sulfur (S8), a waste product of oil refining, with inexpensive allylic monomers. SC-IV has been previously utilized to develop novel precision optical thermoset, termed disulfide glass (DSG), with high refractive index (RI). Owing to the strong optical properties in this material makes it an ideal candidate for photonics devices across the visible and near infrared region. However, the thermoset nature of this material is not solution processable. Addressing this, two methods were developed to limit olefin consumption to produce low conversion, soluble oligomer. DSG from both methods can be used to make polymeric ink, processed into thin-films, and be employed in various photonics devices via photopatterning. Characterization has shown these DSG photoresist provide micron-scale resolution of photopatterned targets. Herein we report this novel photoresist rivals the commercially available materials through utilization of SC-IV a inexpensive and byproduct free step growth polymerization.

Nicolas Ngo – Biochemistry #68

Research Faculty Advisor: Waleed Elhelaly, HMEL: Hypoxic Myocyte-enriched Essential for Life

Fish Scale Analysis with an Electron Microscope

It is now known that adult mouse and human hearts experience modest cardiomyocytes (CMs) turnover, which is mainly mediated by the proliferation of pre-existing CMs, even though the adult mammalian heart is incapable of

meaningfully recovering functionally following substantial CMs loss. This turnover capacity is thought to be mediated by proliferative competency of a specialized cardiomyocyte population, rather than a stem cell. The maintenance and proper function of stem or progenitor cells in various organs depend on the hypoxia inducible factor 1 alpha (Hif-1α) subunit being stabilized, as they live in microenvironments that are relatively hypoxic. We recently identified a rare population of hypoxic/cycling CMs based on stabilization of the oxygen dependent domain (ODD) of Hif-1α. These CMs demonstrated clonal expansion and made a significant contribution to neo-cardiomyogenesis in the adult heart. Additionally, we used RNA-seq to conduct a differential transcriptomic analysis comparing hypoxic ODD CMs with normoxic CMs (1), in order to identify CMs turnover regulators and clarify the mechanism by which these ODD CMs are maintained in a hypoxic/cycling state. Conceivably, these genes may serve as therapeutic targets in order to stimulate adult CMs to proliferate and regenerate the heart. Here we present data on a previously unidentified gene that we named "Hypoxic Myocyte-enriched Essential for Life" (HMEL). We found that HMEL was significantly upregulated by 9.3-fold in the ODD CMs compared to the normoxic ones (1). Despite having 95.8% homology with its human ortholog and being conserved across many species, the mouse gene HMEL's protein sequence showed no homology to any other known genes, according to phylogenetic and homology analysis. While this might indicate HMEL has a distinctive function, it also signifies a challenge to elucidate its function. Preliminary results indicate that HMEL protein expression in the heart follows the transition from the hypoxic/proliferative neonatal heart to the normoxic/non-proliferative adult heart in a descending manner. To better understand HMEL function in vivo, we created a genetic loss of function mouse model. By mating heterozygous knockout mice, we failed to obtain any homozygous pups at birth by genotyping. In addition, the litter size was smaller than expected with an average of 4.5 pups per litter compared to an average of 8 pups per litter when their wild-type (WT) littermates were mated. This suggests that HMEL is a recessive embryonically lethal allele. To determine the approximate timing of embryonic lethality, we created multiple timed crosses between the heterozygous littermates, then collected uteri and dissected conceptuses at different gestational ages starting at E10.5. At E10.5, we could see smaller conceptuses resorbed in the uterus with gross anatomy and LacZ staining, further confirming embryonic lethality. When these conceptuses were dissected, they lacked both yolk sac and embryonic tissue. At E8.5, we were able to find and collect tissue samples from the highly resorbed homozygous knockout embryos using H&E sections, laser dissection and subsequent genotyping, further confirming the recessive embryonic lethality phenotype. In addition, we collected decidua at E6.5 for H&E staining, as well as Ph3 and TUNEL immunofluorescence to further elucidate the lethality phenotype. Our results showed significant anatomically deformed homozygous knockout embryos compared to the heterozygous and WT embryos, a significant decrease Ph3 and an increase in TUNEL staining in homozygous knockout compared to both heterozygous knockout and WT, which in turn showed no significant difference whether histologically or by Ph3 or TUNEL staining. To further characterize homozygous knockout embryos and deduce the precise timing of lethality, we collected and cultured blastocysts from heterozygous knockout crosses at E4.5. We then distinguished homozygous knockouts from heterozygous knockouts and WT by individually genotyping each blastocyst after dark-field microscopic imaging. Our results confirmed that homozygous knockout blastocysts are still viable at this gestational age and have the same histological features as heterozygous knockout and WT embryos. To further investigate the role of HMEL in embryonic stem cells (ESCs), we cultured ESCs from blastocysts from a heterozygous knockout cross. However, only ESCs from heterozygous knockout and WT blastocysts survived, while ESCs from homozygous knockout blastocysts did not. We conclude that HMEL is a recessive embryonically lethal allele where its haploinsufficiency is tolerated and that the time of embryonic lethality is between E4.5 and E6.5, i.e., peri-implantation lethality. We also hypothesize that HMEL is essential for the viability of the ESC, especially during the in vivo transition of the preimplantation embryo from ~8.7% oxygen in the oviduct decreasing to a more hypoxic 1.5–2% oxygen in the uterus (2).

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GO analysis of the pulled down proteins using David's analysis tool revealed an enrichment of biological processes involved in the regulation of RNA binding, RNA processing, mRNA splicing and RNA splicing. Thus, we hypothesize that HMEL may be a novel RNA-binding protein. We used the Antibody IP Validation Kit (Eclipsebio) to confirm the RNA binding ability of HMEL. Briefly, HEK293 cells overexpressing either N-terminally or C-terminally FLAG-tagged HMEL protein were grown to approximately 70% confluence and UV-cross-linked at 400 mJ/cm2. After lysis, cell lysate corresponding to approximately 50 µg of RNA was subjected to partial RNase I fragmentation and immunoprecipitation using α-FLAG magnetic beads. The washed bead fraction was end-repaired and ligated to biotinylated RNA oligo. Biotinylated RNA was separated by 4–12% NuPAGE and detected with streptavidin-HRP. Both N-terminal and C-terminal FLAG-tagged proteins were equally able to bind to RNA. Further experiments will include identifying HMEL binding sites in the transcriptome using eCLIP. In addition, since the time of lethality coincides with the gastrulation process (E6.5), we decided to test the effect of HMEL gain and loss of function on the ESC differentiation process. However, because ESC culture from the homozygous knockout blastocysts is not possible, we decided to isolate and culture primary fibroblasts from F/F and WT mice, then reprogram them into iPSC (using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit) while knocking out and overexpressing HMEL.

Isaac Nicholson – Ecology and Evolutionary Biology #70

Research Faculty Advisor: Scott Bonar, School of Natural Resources and the Environment

Fish Scale Analysis with an Electron Microscope

My project has focused on analyzing the rings found on the scales and fin rays of the desert sucker fish (Catostomus clarkii) using three methods: A regular dissecting scope, a SKIPPY digital camera, and a scanning electron microscope. I have been doing this in order to explore the correlation between the number of rings on an individual specimen's scales and its age, as well as comparing the effectiveness of these three methods of analysis. In doing so, I hope to shed new light on novel methods of fish age determination.

Ronald Palmenberg – Biochemistry; German Studies #77

Research Faculty Advisor: Matthew Cordes, Chemistry and Biochemistry

Key mutations associated with venom recruitment of a recluse spider toxin

Loxoscelism is a dermonecrotic and/or systemic condition in humans caused by the venom from recluse spider bites. The most active ingredient in the toxin cocktail is a group of enzymes, phospholipase D toxins (PLDs). At some point in their evolution, recluse spiders recruited PLDs from non-venom housekeeping enzymes to venomous toxins. Ancestral Sequence Reconstruction (ASR) was utilized to create multiple pairs of pre-venom recruitment and common venom ancestor proteins with the goal of finding the specific sequence changes associated with recruitment. Mutations were identified in two distinct binding pockets: the active site (I246M) and a potential allosteric site (W225T/T233S). The reverse of these mutants were introduced in a venom ancestor, purified and tested with enzyme kinetics and liposome pull-down assays. The retromutation M246I in the active site was found to have increased activity towards all known substrates. The retromutations T225W/S233T in an allosteric site were found to have minor effects on enzyme activity but significantly increased binding towards sphingomyelin-rich liposomes. Both mutations showed some shift in substrate preference towards lipids with ethanolamine head groups over choline head groups. Apparent loss in catalytic activity is surprising during a venom recruitment event. However, a shift away from choline head group binding/catalysis may be explained by the universal presence of sphingolipids with ethanolamine head groups in spider prey. Future studies will focus on the combined effect of mutations at both sites, measuring kinetics, substrate competition and substrate cooperativity.

Delaney Petruzelli – Biochemistry #81

Research Faculty Advisor: Thomas Gianetti, Chemistry and Biochemistry

Pendant Arm and Bridging Atom Variations in Carbenium Ions: Synthesis, Properties, and Applications.

Triaryl carbenium ions, such as AzaDiOxaTriAngulenium (ADOTA) and DiMethoxyQuinAcridinium (DMQA), are stable carbocations that have demonstrated unique applications in red-light-mediated photoredox catalysis and symmetric organic redox flow batteries. The photophysical and electrochemical properties of these carbenium cores can be tuned through various modifications. Herein, we explore different variations of pendant arms around the carbenium core, as well as different bridging atoms (i.e. nitrogen vs. oxygen). We successfully introduced a pyrene pendant arm and a bipyridine pendant arm. The pyrene arm unlocks access to an extended conjugated system via ring closure with the core, while the bipyridine arm enables metal binding. We present their synthesis, photophysical and electrochemical properties, and discuss their potential applications in future research.

Natalie Rawlings – Molecular and Cellular Biology; Biochemistry #73

Research Faculty Advisor: Andrew Capaldi, Molecular and Cellular Biology

Characterizing the dynamics of a novel regulator of nutrient signaling and transport in the TORC1/SEAC interactome

The Target of Rapamycin Complex 1 (TORC1) is a key regulator of eukaryotic cell growth and metabolism. Although this 2-billion-year-old, highly conserved protein complex is a central control hub for growth, proliferation, autophagy, and stress response, many mechanisms of its regulation remain unclear. Mutations in proteins involved in the TORC1 pathway are correlated with numerous human diseases, including cancer, diabetes, and epilepsy, and as such, building a comprehensive, quantifiable model of the nuanced regulation of this pathway will lay the foundation for developing novel TORC1-based therapeutics. Previous work in Saccharomyces cerevisiae has highlighted the role of the vacuolar GPCR-like protein Ait1 in TORC1 regulation via interactions with the small GTPases Gtr1/2. In characterizing the behavior of Ait1, the protein Vsb1 was identified as a potentially novel regulator of the TORC1/SEAC interactome of similar significance to Ait1. Building upon this foundation, this research focuses on Vsb1, a vacuolar arginine transporter with secondary activity in histidine and lysine transport, and its impact on TORC1 dynamics. Preliminary findings suggest a functional interaction between Vsb1 and Ait1 in amino acid signaling, with significant overlapping response patterns during histidine starvation. Here, we extend methodologies from earlier studies on Ait1, examining TORC1 activity in various mutant strains under numerous starvation conditions. This work aims to elucidate whether Vsb1 exerts its regulatory effects on TORC1 through either a direct or indirect interaction with Ait1 and other intermediate pathways, and to determine how Vsb1 itself is regulated, ultimately to create a more comprehensive model of TORC1 regulation.

Erin Schuette – Biochemistry #64

Research Faculty Advisor: Jared Churko, Cellular and Molecular Medicine

Promoting embryonic stem cell derived cardiomyocyte maturity by gene editing sarcomeric proteins

The research of cardiovascular disease mechanisms involving cardiac muscle cells has always been difficult given that adult cardiomyocytes (CMs) do not proliferate. Thus adult CMs cannot be harvested from tissue, reproduced in culture, and experimented on in vitro. However, constant development in stem cell research has created new opportunities for discovery in the cardiovascular field. Human embryonic stem cell derived cardiomyocytes (hESC-CMs) provide an abundant and easily attainable source for modeling cardiac muscle cells in healthy and diseased states. Yet, there are many criticisms regarding the accuracy of hESC-CMs in modeling adult cardiomyocyte phenotypes. This project proposes that knockout of three fetal sarcomeric protein encoding genes will induce increased expression of genes that encode for the adult protein isoforms. Therefore, a more

phenotypically mature hESC-CM line would be created with observable differences in contraction and ATP metabolism. Contraction metrics analysis revealed a lower BPM rate and increased contraction duration in fetal knockouts. However, no significant change in ATP metabolism was observed, suggesting this measurement was unrepresentative of contraction behavior differences between cell lines. Future experimentation with calcium kinetics could help connect contraction observations to molecular coupling with calcium influx and ATP hydrolysis between the myosin and actin filaments.

Alexis Schwartzberg – Biochemistry #95

Research Faculty Advisor: Juliana Gil-Loaiza, School of Natural Resources and The Environment

Microbial Adaptation to Elevated Hydrogen: Soil as an Atmospheric H_2 Sink

The exposure of soil located near hydrogen production and refueling stations to elevated hydrogen (H_2) levels was investigated to study the effect on microbial adaptation and hydrogen consumption efficiency to mitigate the negative impacts of excess hydrogen emissions. Through leaking and venting that occurs during use and distribution processes of hydrogen at the stations, hydrogen is emitted into the atmosphere. This contributes to global warming effects since interactions with methane, ozone, and water can occur, allowing these greenhouse gases to persist in the atmosphere longer. Soil microbes are a key regulator of hydrogen emissions, absorbing about 80% of atmospheric hydrogen. Hydrogenases are enzymes that allow microbes to metabolize hydrogen. High-affinity hydrogenases utilize hydrogen at low concentrations, while low-affinity hydrogenases require higher concentrations to utilize hydrogen effectively. We hypothesized that soils that have been introduced to elevated hydrogen would demonstrate higher levels of hydrogen consumption, because the microbes would have adapted to metabolize hydrogen effectively. To test this hypothesis, soils taken from three hydrogen production and refueling sites will be used for soil jar incubations and exposed to 50 ppm hydrogen. The hydrogen concentration in the jar will be measured every 12 minutes for a total of 48 minutes. We found in this study that the site had no impact on hydrogen consumption, and the hydrogen fluxes were lower than expected. However, this could be due to the intermittent exposure of soils to hydrogen, not allowing them to accumulate hydrogen-oxidizing bacteria and the activation of low-affinity hydrogenases that are ideal for metabolizing elevated hydrogen levels. Previous research highlights that persistent exposure allows microbes to evolve specialized ways of handling hydrogen overtime. It is possible that the soil in this experiment only contained high-affinity hydrogenases that deactivated in the presence of elevated hydrogen which potentially limited the uptake of the soils. We aim to test the effects of longer and continuous exposure of soil to higher elevated hydrogen levels and perform an analysis of the types of microbes and enzymes found in our samples, which will give a greater understanding of how microbes adapt to different hydrogen concentrations and how it affects their sink capacity.

Trin Šutalo – Ecology; Evolutionary Biology #84

Research Faculty Advisor: Vlad Kumirov, Chemistry & Biochemistry

Structure of Formazan Derivatives using 2D NMR Spectroscopy

Formazans are commonly used as dyes and metal chelators. The structure of formazan derivatives was determined using 2D NMR spectroscopy. These structures vary depending on aromatic substituents and solvent used. NMR spectroscopy is very useful in studying dynamic properties of formazans, such as chemical exchange between different structural forms.

Kai Walsh – Chemistry #80

Research Faculty Advisor: Dominic McGrath, Chemistry and Biochemistry

Studying verdazyl radical single molecular junctions: Synthesis and applications

We synthesized four series of verdazyl radical derivatives bearing thiomethyl linkers. This approach enables the exploration of radical single-molecule junctions as a new strategy to overcome limitations in the current-carrying

capacity of Au mechanically controlled break junctions (MCBJs), enhance molecular conductance, and create tunable spin filters. The four verdazyl radical series differ in their functionalization positions on the verdazyl core, allowing us to tailor the ionization energy /electron affinity range and exert better control over the SOMO/SUMO gap. This project focuses on the synthesis of one verdazyl radical variant from the fourth series of derivatizations through a multistep synthesis starting with 4-(methylthio)aniline. In this fourth series, various benzaldehydes were used in the hydrazone step, and different benzyl bromides were employed for the final ring closure, which was followed by an in-situ oxidation.

Charlie Woodring – Biochemistry #96

Research Faculty Advisor: Laura Meredith, School of Natural Resources and the Environment

Microbial Responses to Hydrogen Exposure Near Fueling Stations: Implications for Environmental Impact

Hydrogen (H2) energy fuel cells have flourished as an alternative energy source. Currently, the recharging of H2 cells utilizes large, cooled tanks of H2 creating a point source of H2, potentially increasing surrounding atmospheric concentrations contributing to global warming. Increased exposure to H2 could lead to changes in the microbial makeup of soil, particularly an increase of H2 oxidizing activity. These microorganisms use H2 as an electron donor within many processes of the carbon cycle allowing for H2 lost during the refueling process to be consumed for metabolic purposes.

In this study, we examine the potential microbial capacity within different soil samples when historically exposed to different hydrogen concentrations from venting events to find a relationship between the duration and proximity of the soil from a point source and the ability to uptake H2 from the atmosphere. In the laboratory, we incubated soils from two sites, whose historical H2 exposure varied from 1 year to 10 years, at different distances downwind of the primary H2 fueling stations and compared them to a control site. We exposed the incubation jars to atmospheric H2 concentration for 3 minutes and used a reducing gas detector chromatograph to measure soil H2 uptake every 10 minutes. Results from this study will inform the understanding of the potential capacity of soil microbes to respond to increased exposure of H2 allowing us to make recommendations on the location and management of these H2 sources to mitigate environmental impacts.

Forrest Zepezauer – Biochemistry; MCB; Ecology & Evolutionary Biology #74

Research Faculty Advisor: Polly Fordyce, Cellular and Bioengineering and Genetics

Genetic Code Expansion with High-Throughput Microfluidic Enzyme Kinetics

Enzymes are catalytic biomolecules capable of extreme efficiency and specificity for their target substrates. The complex kinetic and thermodynamic factors that promote such efficiency are governed by interactions between residues both proximal and distal to the active site. An emerging strategy for decoding sequence-function relationships is strategic incorporation of noncanonical amino acids (ncAAs) that are not part of the twenty standard amino acids used in protein synthesis. The diverse chemical properties of ncAAs make them promising functional probes that can uncover information about folding, reactivity, and beyond. However, methods for incorporating ncAAs offer insufficient yield for quantitative studies of protein function, and drawing conclusions requires ensuring that ncAAs are faithfully incorporated at the correct locations. A novel method from the Fordyce Lab for probing sequence-function relationships is High-Throughput Microfluidic Enzyme Kinetics (HT-MEK), which allows for simultaneous in vitro expression, purification, and kinetic characterization of up to 1,568 variants per experiment. HT-MEK uses minimal reagents and generates attomole amounts of protein per variant, making it a promising candidate for alleviating the bottlenecks that have traditionally limited ncAA studies. Here, we demonstrate in vitro protein expression on HT-MEK using genetic code expansion (GCE) to incorporate selected amino acid substitutions at targeted sites via suppression of the amber stop codon, ‘TAG’ (called ‘amber suppression’). To establish the feasibility of this approach, we first aimed to recapitulate the effects of gold-standard site-directed mutagenesis (SDM) through GCE. We generated canonical amino acid (cAA)-tRNA pairs of phenylalanine, alanine, and glycine using an engineered aminoacyl-tRNA synthetase and expressed hAcyP2 constructs with cAAs incorporated at select residues via GCE. To quantify the efficiency with which these

different cAAs were incorporated, we quantified catalysis for each variant and compared activity to SDM variants bearing the same substitutions. Proteins expressed using GCE expressed at the same levels as their WT and SDM counterparts and were similarly active (kcat and KM within 26.6% and 13.9% of WT, respectively), establishing the feasibility of this approach and demonstrating efficient incorporation of the correct amino acids via GCE. These results establish the feasibility of combining GCE with high-throughput microfluidic enzyme expression, purification, and deep functional characterization, laying the foundation for future work using ncAAs to dissect mechanisms of catalysis and assess broader aspects of protein function such as the impacts of post-translational modifications.


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