The ACS-SA 19th Annual Undergraduate Research Symposium

Abstracts

Department of Chemistry and Biochemistry
University of California, San Diego

Thursday, May 15, 2025
4PM – 7PM

Keynote Speaker:

Dr. Lisa Jones

Lisa M. Jones grew up in Mt. Vernon, NY. She did her undergraduate degree in Biochemistry at Syracuse University and her PhD in Chemistry at Georgia State University. She did postdoctoral training in structural virology at the University of Alabama-Birmingham and in MS-based protein footprinting at Washington University in St. Louis. She started her first faculty position at Indiana University-Purdue University Indianapolis (IUPUI) in 2012. In 2016, she moved to the University of Maryland Baltimore. In 2022, she moved to the University of California San Diego as the Chancellor's Associates Endowed Chair in Chemistry and Biochemistry.

Dr. Jones' research is focused on developing MS-based structural biology methods for studying complex model systems such as cells, spheroids, and C. elegans and using these methods to study protein conformations and interactions in their native cellular environment. She also has a passion for increasing diversity in STEM and participates in several outreach initiatives to achieve this. In 2019, she received the Biophysical Society Biopolymers in Vivo Subgroup Junior Faculty Award which recognized her success as a young investigator.

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Impacts of BCR-ABL Mutations on Cell Proliferation and TKI Treatment in CML

***Elina Adenyayeva*, Tara Shammas**
Principal Investigator: Dr. Daniel J. Donoghue

The BCR-ABL fusion protein results from a translocation between Chromosomes 9 and 22, and plays a critical role in the development of chronic myeloid leukemia (CML). This fusion causes an overactive tyrosine kinase, driving uncontrolled and excessive cell proliferation, making it a target for therapeutic interventions. To combat this, many tyrosine kinase inhibitors (TKIs) have been developed, with Imatinib being the first. However, the emergence of mutations within BCR-ABL, particularly in the kinase domain, has led to resistance against many first and second generation TKIs, complicating treatment strategies. In this study, different mutated BCR-ABL constructs were transfected into 32D cells to generate a stable cell line expression the mutated BCR-ABL and wild-type BCR-ABL. The MTT assay was utilized to assess the functional consequences of the various BCR-ABL constructs in 32D cells in the presence and absence of Interleukin-3 (IL-3), a growth factor needed for the development and growth of 32D cells. The assay allowed for the quantification of cell proliferation in response to both TKI treatments and mutation-specific alterations in BCR-ABL. By examining the viability of cells expressing a variety of BCR-ABL mutants, the impact of these mutations on cell survival can be uncovered, along with new therapeutic targets to be developed to target BCR-ABL.

<u>Investigating NFkB with its intrinsically disordered domain (TAD)</u>

***Huishan (Natalie) Chen*, Tristan Gunther**
Principal Investigator: Dr. Elizabeth A. Komives

We rely on transcription factors to bind to DNA and properly turn on specific genes in response to outside signals. One of these transcription factors, known as NFkB, plays a critical role in our inflammatory response after its activation by external stress signals. However, NFkB shows complex DNA binding behavior. We hypothesize that the key to NFkB's function stems from its multi-domain nature; in some cases, the intrinsically disordered transcription activation domain (TAD) of the main NFkB heterodimer, known as RelA/p50, may interact with other domains within RelA/p50 and affect their DNA-binding function, or with other proteins, or both. To probe these interactions we are using targeted crosslinking. This approach involves creating an amber codon at the sites where we will then introduce a photoactivatable crosslinker using unnatural amino acid incorporation (mutation of key charge). The goal of this project is to understand the dynamics of the folding of our protein and how the intrinsically disordered TAD impacts DNA binding affinity. Researchers have been missing this interaction of the protein because it was thought to not have a major impact on DNA binding and they have only studied the folded region or the disordered region separately. The discovery of potential interactions between domains pushed us to conduct experiments to learn more about the interaction of the TAD region with RelA/p50 protein and its impact on DNA binding.

<u>Size Selectivity by Phase-Separating Proteins of the Early Secretory Pathway</u>

Samantha Cheung, **Savannah Bogus**
Principal Investigator: Dr. Andreas Ernst

The early secretory pathway, comprised of the endoplasmic reticulum (ER) and the Golgi apparatus, is essential for transporting newly synthesized proteins and lipids. However, the mechanisms governing bidirectional transport between the ER and Golgi, and cargo sorting at the trans-Golgi network (TGN), remain unclear. Trk-fused gene (TFG) forms a porous, liquid-like condensate between the ER and Golgi that acts as a molecular sieve. With a size cutoff around 250 kDa—roughly 8 nm—it permits access to anterograde COPII coats while excluding larger components like retrograde COPI coats and ribosomes (~20 nm). In vitro assays confirmed that fluorescently labeled ribosomes are excluded from TFG condensates, supporting this size-based filtering model. Similarly, trans-Golgi-resident golgins—coiled-coil tether proteins including GCC88, golgin-97, GCC185, and golgin-245— form a ribosome-exclusion zone and may contribute to protein sorting. TGN golgins can undergo liquid-liquid phase separation and form two-dimensional, de-mixed condensates on model membranes, suggesting a role in Golgi self-organization and cargo sorting. Recent studies propose that apical and basolateral membrane proteins are sorted by size at the Golgi. To explore this, recombinant GCC88 and golgin-97 were purified and tested for phase separation, demixing, and selective cargo sequestration, offering new insights into the spatial and functional organization of the Golgi.

<u>In vitro studies of co-transcriptional mitochondrial RNA processing</u>

***Shannon Cole,* Sean Reardon**
Principal Investigator: Dr. Tatiana V. Mishanina

Mitochondria are eukaryotic cellular organelles that are responsible for producing the majority of a cell's ATP supply via electron transport through oxidative phosphorylation (OXPHOS). The protein complexes for OXPHOS are generated through a coordinated effort of nuclear encoded gene expression and the expression of thirteen proteins encoded on mitochondrial DNA (mtDNA). Via the nuclear-encoded mitochondrial specific proteins: Mitochondrial Transcription Factor A (TFAM), Mitochondrial Transcription Factor B2 (TFB2M), Mitochondrial RNA polymerase (POLRMT), and the Mitochondrial Transcription Elongation Factor (TEFM), the mitochondria transcribe two ribosomal RNAs (rRNAs), 22 transfer RNAs (tRNAs), and thirteen OXPHOS complex protein coding genes. The rRNA and tRNA genes are heavily modified in order to achieve active 3D-conformations. We are hypothesizing that in order to be correctly modified, these highly structured RNAs must be modified co-transcriptionally. One of the topics of interest includes the length of pre-tRNA and if RNA maturation complexes are efficiently recognizing and cleaving the tRNAs regardless of its nascency. Further, we are hypothesizing that transcriptional pausing coordinates these modification events, leading to correct RNA folding. We will be exploring these hypotheses via cleavage assays testing the extent of RNA maturation complex capabilities and in-vitro mitochondrial transcriptional assays to determine potential pausing sites on the mitochondrial genome.

<u>The influence of binding partner Shugoshin on HP1α liquid-liquid phase separation</u>

Tonkhla Dankul, **Rushabh Bhakta**
Principal Investigator: Dr. Galia Debelouchina

Heterochromatin protein 1α (HP1α) is an essential component in the organization of chromatin into the more compact and transcriptionally silenced heterochromatin state. This is in part due to HP1α's ability to undergo liquid-liquid phase separation and form liquid like droplets that selectively manage which other proteins can enter and interact with chromatin inside the droplet. It has also been discovered that HP1α's propensity to phase separate is modulated through interactions with protein binding partners (PBPs). Shugoshin (Sgo1) is a protein thought to help maintain centromere stability during mitosis, and it is also a known PBP of HP1α, however, the details behind its interaction with HP1α and influence on phase separation have not been elucidated. Here, I plan to use various experimental approaches to study the interactions between full length HP1α and a segment of Sgo1. The effects of Sgo1 on HP1α's ability to phase separate will be directly observed through the use of fluorescence microscopy. This will be paired with tryptophan fluorescence spectroscopy to determine the binding affinity between HP1α and Sgo1. Finally, solution nuclear magnetic resonance (NMR) spectroscopy experiments will be performed to capture the interactions between the two proteins. Through these experiments, I hope to learn Sgo1's role in HP1α liquid-liquid phase separation and how this connects to Sgo1's involvement in centromere stability.

<u>Investigating the role of Flavodoxin II in the reactivation of O_2-protected nitrogenase</u>

Peter Dukakis, **Sarah Narehood**
Principal Investigator: Dr. Mark Herzik, Dr. Akif Tezcan

Nitrogenase is the only known enzyme capable of reducing atmospheric nitrogen (N_2) to biologically available ammonia (NH_3) under ambient conditions, a process vital for life and with agricultural and industrial importance. Unlike the industrial process used for nitrogen fixation which has negative impacts on the environment, nitrogenase fixes nitrogen under ambient conditions and with little environmental costs. Many aspects of the catalytic mechanism for nitrogenase remain unanswered. A recent discovery in the field showed that the oxygen sensitive metal clusters of nitrogenase systems are conformationally shielded by the association of a Shethna (FeSII) protein under oxidizing conditions. Our work investigates the role of Flavodoxin II (FldII), a hypothesized reductant of this FeSII-nitrogenase complex that may promote decomplexation and the reactivation of catalytic activity. To explore this, we cloned and optimized a procedure for expressing Azotobacter vinelandii FldII in E. coli. We then purified FldII using anion exchange and size exclusion column chromatography. FldII contains the cofactor flavin mononucleotide which contributes to its redox properties. We used UV/Vis spectroscopy to analyze the apo/holo ratio of the purified FldII as the apo and holo forms of the enzyme each contain a distinct UV/Vis signal. To begin investigating our hypothesis, we used electron paramagnetic resonance spectroscopy (EPR) to show that electron transfer between FldII and isolated FeSII is possible. Further experiments are planned including analytical ultracentrifugation (AUC), Protein Film Electrochemistry (PFE), as well as more EPR and UV-Vis studies to further explore our hypothesis.

<u>Mimicking of Neurodegenerative Diseases through Saporin Immunotoxin Neuronal Lesioning</u>

***Joe Eastham,* Patrick Shramm, Raschel Bouajram**
Principal Investigator: Dr. Leonardo Ancheta

Saporin, a protein derived from Saponaria Officinalis, is a cytotoxic protein that causes irreversible inhibition of protein synthesis from the removal of an adenine base from the ribosomal RNA of the eukaryotic ribosome. The absence of the binding chain makes Saporin incapable of entering cells on its own and this feature allows it to be used as a potent and effective payload. When saporin is linked to a targeting agent, which can be anything that binds to a cell surface receptor (antibody, lectin, peptide, etc.) saporin acts as a molecular scalpel and can eliminate specific cell types in a way that can mimic diseases, e.g. Alzheimer’s or Parkinson’s. By replicating symptoms or behaviors of these diseases researchers can study 1) the disease itself, 2) drugs that may treat that disease, and/or 3) the cell populations responsible for the impacts of those diseases. By manipulating the targeting agent, the location of injection, and dosage of the saporin immunotoxin in an animal model, a researcher’s specific goal can be achieved with respect to Alzheimer’s Disease, Parkinson’s Disease, and more.

Structural Characterization of the Novel Obelisk-Oblin RNP in the Human Microbiome

***Deidre Gorian,* Alexa Perez**
Principal Investigator: Dr. Navtej Toor

In recent studies, a novel family of RNA with viroid-like characteristics has been identified in human gut metatranscriptomic data by Zheludev et al., Cell 2024, named the 'Obelisks.' The Obelisks were categorized by three criteria: having circular RNA genomes of approximately 1000 nucleotides, a distinctive rod-like predicted secondary structure, and the ability to code for any member of the associated novel protein family, named the Oblins. The Obelisks and the encoded Oblins lack any structural or sequence similarity to any known phylogenetic group. However, sequencing analyses found the Obelisks to be prevalent in humans, identified in 9.7% of the study's 472 total donors. However, minimal information is known about the Obelisks' functional significance or effects on host fitness.

We have purified several Obelisk strains and their associated Oblin proteins to conduct structural and functional studies of the Obelisk RNA. Cryo-EM studies were performed to analyze the proposed rod-like structure of the Obelisk. RNA-protein binding assays were also conducted to assess the formation of an Obelisk-Oblin ribonucleoprotein (RNP) complex.

We have investigated the replicative mechanism of the Obelisk RNA, proposed to follow a viroid-like rolling-circle mechanism in the host cell, using its encoded proteins to repurpose cellular RNA polymerases for RNA-dependent RNA replication. Now we propose a method to study the behavior of the circularized Obelisk RNA in vivo and its retention by a model bacterial system.

<u>Elucidating the structure specificity of Sesquiterpene cyclase in the mechanism of capnellene biosynthesis</u>

***Yantong Guo*, Immo Burkhardt**
Principal Investigator: Dr. Bradley Moore

Terpenoids are one of the largest family of natural products in the world with over 95,000 known compounds, which have been widely used in pharmaceuticals, food and agricultural industries due to their unique biological properties and chemical reactivity. Capnellene, isolated from the marine soft coral, has shown potential as a therapeutic agent for inflammatory diseases. Herein, we investigate the structural and mechanistic basis of capnellene biosynthesis by exploring the role of key enzymatic residues in sesquiterpene cyclase. By introducing targeted amino acid substitutions—selected for their polarity and steric bulkiness—we reveal how specific residue modifications can influence enzyme function and the overall cyclization pathway from precursor farnesyl diphosphate (FPP) to capnellene. Our findings provide new insight into sesquiterpene cyclase's structure-function relationship and open up avenues for sesquiterpene cyclase engineering to generate novel terpenoid scaffolds with improved therapeutic potential.

<u>Biochemical Characterization of a Flavodiiron Protein from Histamonas meleagridis: An Alternative Pathway for Mitochondrial Disease Therapeutic Treatment</u>

Victoria Heitzmann, **Subrata Munan**
Principal Investigator: Dr. Valentin Cracan

Mitochondrial diseases affect over 20,000 people in the US every year and are characterized by a buildup of reactive oxygenated species (ROS). Electrons passed down the electron transport chain (ETC) are not passed to the final electron acceptor, causing a buildup of oxygen in the mitochondria, which can generate reactive oxygenated species (ROS) and damage the cell through oxidative stress. We seek to explore an alternative redox pathway via the FDP protein found in H. meleagridis (HmFDP) to reduce oxygen to water. The overexpression of proteins in E. coli and their subsequent purification, followed by a biochemical characterization, including activity assays, is an important methodology which can be applied to multiple systems. We present a pipeline on how we obtain an FDP protein for biochemical characterization. The conclusions of this study are applicable towards ongoing efforts in our laboratory towards next generation mitochondrial disease therapeutics. Herein, a plasmid encoding HmFDP was transformed into E. coli, and overnight culture was grown in LB medium and used to prepare main culture when bacteria are grown in Terrific Broth (TB) medium. We use affinity NiNTA, ion exchange S15Q chromatographies, as well as gel filtration using Superdex 200 to purify HmFDP. All steps of purification are monitored using SDS-PAGE. We characterize and test enzyme activity using UV-Vis spectrophotometry. Resulted protein sample was >95% pure based on SDS-PAGE analysis as well as biochemical characterization. Further kinetic studies are planned to study the in-depth catalytic mechanism of HmFDP.

Gene Silencing of Cathepsin B to Ameliorate Cell Death Neurodegeneration

***Madison Heng*, Sonia Podvin**
Principal Investigator: Dr. Vivian Hook

Cathepsin B is a powerful lysosomal protease that is upregulated in neurologic conditions causing behavioral dysfunction and neuropathy. Gene knockout of cathepsin B has shown improved memory and behavioral deficits in models of Alzheimer's disease and traumatic brain injury. We are evaluating the hypothesis that cathepsin B participates in cell death neurodegeneration in neuronal and microglia cells. To investigate the role of cathepsin B, we are developing in vitro conditions for the siRNA:peptide silencing of cathepsin B in mouse neurons. Through various experiments, a specific siRNA sequence has been identified which has shown to be most effective in reducing mouse cathepsin B mRNA. The siRNA and transfection reagent are co-incubated to form the lipid nanoparticle-siRNA complexes, which is added to the cultured cells and uptaken into the cell where it will trigger mRNA degradation. The most effective siRNA sequence has been shown to reduce cathepsin B levels by approximately 70%. Effectiveness of cathepsin B gene silencing was measured using a cathepsin B enzyme-linked immunosorbent assay conducted in triplicate and compared to a negative control. In conjunction, protein assays and lactate dehydrogenase assays were conducted to standardize the measurements. Gene silencing in neuronal and glial cell cultures will assess the role of cathepsin B in stress-induced cell death. Overall, results show that gene silencing of cathepsin B is achieved by siRNA, and will be used to assess the role of cathepsin B in neurodegeneration that occurs in Alzheimer's disease and traumatic brain injury.

Compound i9 and i12 upregulate memory-like markers in human and mouse T-cells

***Kaitlyn Kim,* Ke Qin**
Principal Investigator: Dr. Peng Wu

The development of T-cell exhaustion in the tumor microenvironment limits the efficacy of CAR T-cell therapies. For example, terminally exhausted T-cells are dysfunctional and exhibit attenuated effector function, potentially affecting the anti-tumor efficacy of CAR T-cells. In addition, T-cell exhaustion contributes to poor persistence in vivo and makes CAR T-cell therapies lose their long-term efficacy. As a result, some adoptive transferred CAR T-cells fail to clear tumors effectively in vivo. Therefore, a major area of research in the Wu lab has been to identify and utilize small molecules to prevent T cell exhaustion and maintain a more stem-cell-like feature to achieve durable anti-tumor responses for CAR T-cell therapy. Previous chemical screens in the Wu lab have identified two small molecules that are able to preserve memory-like phenotypes: i9 and i12. In this poster, I present how the identified compounds can decrease inhibitory receptors like PD-1, Tim-3, and CD39, and increase memory-associated markers including cell surface markers CD62L, CD45RA, and an intracellular transcription factor TCF-1 as demonstrated by flow cytometry data in both mouse and human models. Consistent with the phenotype data, I also show the cytokine production of compounds-reprogrammed T cells in response to antigens in vitro. These findings are significant and serve as a starting point for improvements for additional improved CAR T-cell research.

<u>The effect of novel small non-coding RNAs on pH-responsive riboswitch controlled alx gene in Escherichia coli</u>

***Kim Lam*, Ravish Sharma**
Principal Investigator: Dr. Tatiana Mishanina

Manganese ion (Mn2+) is vital in helping enzymes in cellular functions by acting as a cofactor and protecting against reactive oxygen species. Too much Mn2+ can be toxic to the cells so there are regulatory mechanisms that help in controlling the amount of Mn2+. Commensal bacteria, such as Escherichia coli (E. coli) has the riboregulatory processes to protect from excess Mn2+: the 5' untranslated (UTR) region of mRNA that binds to Mn2+ and promotes the expression of downstream genes. These 5' UTRs are called Riboswitches. Two genes that are controlled by Mn-responsive riboswitches in E. coli are alx and mntP: these genes encode for Mn^{2+} exporters proteins to detoxify excess Mn2+. The expression of alx gene is pH responsive too. Mishanina lab has recently established that the riboswitches-mediated regulation of alx are also controlled by small noncoding RNAs (sRNAs). The precise mechanism by which sRNAs regulate the activity of Mn2+ sensing riboswitches of alx is unclear. I am going to transcribe these novel sRNA by T7-RNA polymerase and purify them. Later the effect of these novel sRNAs will be tested on alx transcription in vitro.

<u>Ki-67 promotes HP1α phase separation and regulates heterochromatin domains in neurodevelopmental disorders</u>

Donald Le, **Lannah Abasi, Challana Tea, Galia Debelouchina**
Principal Investigator: Dr. Cole Ferguson

Neurodevelopmental disorders frequently arise from mutations in chromatin-associated genes, with the ANAPC neurodevelopmental disorder, caused by the loss of the APC7 subunit of the ubiquitin ligase anaphase promoting complex (APC/C), an emerging example. Ki-67, one of the proteins under the APC/C's regulatory purview, consequentially accumulates in constitutive heterochromatin (cHC). Normally, Ki67 associates with cHC and HP1α, a constituent protein of cHC influencing nucleosome compaction and liquid-liquid phase separation (LLPS). Given the prevalence of chromatin dysregulation in neurological diseases, we investigated Ki67's role in heterochromatin organization using reconstituted nucleosome arrays with histone octamers under varying concentrations of Ki-67, phosphorylated Ki-67 (pKi-67), HP1α, phosphorylated HP1α (pHP1α), and specific epigenetic markers. Turbidity assays quantified phase separation extent, while fluorescence photobleaching recovery assays assessed condensate fluidity. In addition, we continued our examination of the effects of perturbations of Ki-67 levels in a novel Ki67 knockout mice model. Our findings demonstrate that Ki67 significantly influences heterochromatin phase separation and cHC compartmentalization, particularly by enhancing chromocenter size. In vitro, Ki67 enhances HP1α-mediated heterochromatin condensation, with pKi-67 promoted condensates having increased fluidity. In vivo, Ki67 knockout mice exhibit cerebellar hypoplasia and growth delay, possibly due to genomic instability and mitotic DNA damage in Ki-67's absence. These findings establish Ki-67 as a crucial regulator of heterochromatin PS dynamics, with its dysregulation—either through overexpression or complete loss—impacting chromatin organization.

Assembly and Modification of a New Confocal Microscope

Issak Loreto
Principal Investigator: Dr. Chris Obara

Since the advent of green fluorescent protein technology by Tsien and colleagues, fluorescence microscopy has emerged as the primary means of identifying specific protein locations within living cells. Traditional widefield techniques, while fast, often suffer from poor signal to noise or high background as a result of nonspecific activation of fluorescent molecules far from the central focal plane of the system. Photons released from these distant planes enter the optical path out of focus, and their origin in the sample is difficult to ascertain. Since the 1950s, confocal microscopy has emerged as the tool of choice to address this limitation. In this approach, a conjugate (Fourier) plane is generated with a set of lenses, the central point of which is passed through a small "pinhole", which blocks light from out of focus planes. The Obara Lab has recently acquired a used scanning point confocal with resonant scanners. In this poster, I will show the assembly, operation, and function of the parts of this confocal microscope, which I have assembled as part of my research. Using this scope, we show high speed recordings of organelle movement, reshaping, and responses to several perturbations including rapid depletion of cellular calcium stores. This tool promises to be useful for new questions in cell biology and biochemistry that must be performed at their location in living cells.

<u>Evaluating FAS-II Dehydratase-Acyl Carrier Protein Complexes from Mycobacterium tuberculosis using Cross-Linking Probes</u>

***Donovan O'Connell, Michael McMaster*, Desirae Mellor**
Principal Investigator: Dr. Michael Burkart

Mycobacterium tuberculosis (Mtb) is currently the leading cause of death from a single infectious agent. The pathogenicity of Mtb and the increasing frequency of drug-resistant infections are in part due to their cell wall which contains mycolic acids (MA). MA are derived from very long-chain fatty acids that are produced via the type II fatty acid biosynthetic pathway (FAS-II). FAS-II contains the target of the first-line tuberculosis drug, isoniazid, but drug resistance has made finding a new FAS-II targeting drug a high priority. Mtb relies on an acyl carrier protein, AcpM, to shuttle the substrate to a suite of partner proteins to be iteratively modified and elongated. The protein protein interactions (PPI) between AcpM and its partner proteins are essential for the regulation of FAS-II, however these interactions are poorly understood. The structural resolution of AcpM-partner protein complexes are instrumental in elucidating targetable PPI, though capturing these interactions has remained difficult. Cross-linking probes that mimic the native substrate have been developed to covalently bind to specific partner proteins, trapping regulatory PPI in the bound complexes. Dehydratases (DHs) are responsible for the formation of the reducible trans-2-enoyl bond from the 3-hydroxyacyl-AcpM substrate. In Mtb, this reaction is catalyzed by enzymes HadAB and HadBC. Through the use chemoenzymatic crosslinking methods, here report progress toward the generation of the tubercular DH, HadAB and HadBC, and their applications towards crosslinking studies with AcpM.

<u>Computational Methods for Azoreductase Enzyme Discovery</u>

***Maeve O'Connor*, Javier Espinoza, Erika Garay**
Principal Investigator: Dr. Adrian Jinich

The azoreductase enzyme family is responsible for the reduction of azo bonds, which is a double bond between two Nitrogens and their respective R groups. Using the protein language model (PLM) ESM-2, an interactive, 2-dimensional visualization of 1,129 orphaned NAD(H)/NADP(H) dependent azoreductases from the uniprot database was created, and 24 enzymes from different clusters were selected. These 24 enzymes were made into recombinant plasmids where the proteins were purified and underwent spectrophotometric assays against a panel of azo dyes, as these dyes had the purpose of mimicking the proteins' natural substrate. From these assays, one protein became of particular interest (azoreductase #28) due to its high activity compared to the rest of the proteins. To find other enzymes with similar or better activity as azoreductase 28, we made a "close up" secondary search into the family it belongs to, which is the PF03358 family. In this secondary search, a sequence similarity network (SNN) and PLM were both used to find new enzymes to test for their catalytic activity. The secondary search revealed additional enzymes with equal or better activity than azoreductase 28 against the panel of azo dyes. Another aspect of this project investigates which is a better method to guide the discovery of enzymes of interest: sequence similarity networks or protein language models.

<u>Mechanism of Riboswitch pH Response</u>

***Avery Ontiveros*, Danea Palmer**
Principal Investigator: Dr. Tatiana Mishanina

RNA-based gene regulation is essential for most cellular processes, and riboswitches in bacterial cells are an important example of such regulation. Riboswitches are mRNA elements that undergo conformational changes to promote or inhibit the expression of a downstream gene. There are two manganese-sensing riboswitches in E. coli that control manganese transporter proteins, alx and mntP. The alx riboswitch also responds to changes in intracellular pH and is the only known riboswitch to respond to these two cellular stimuli. The alx riboswitch has a significantly increased response to Mn2+ at alkaline pH. My project aims to investigate whether the mechanism of this unique pH response is sequence dependent. To achieve this, I perform molecular cloning to swap the Mn2+ binding site sequences of alx and mntP. To assess the pH response of these mutated riboswitches, I perform in vivo β-Galactosidase assays to quantify the translation of the mutated alx and mntP riboswitches under high pH conditions. This work is key to understanding if the mechanism of alx riboswitch pH response is sequence or structure dependent.

<u>Investigation of Dapoxyl Probe's Loading Mechanism onto Carrier-Partner Protein Complex</u>

***Kyle Rich*, Matthew Miyada**
Principal Investigator: Dr. Michael Burkart

Carrier protein-dependent pathways are biosynthetic pathways that depend on carrier proteins to synthesize various biomolecules. We are interested in this class of pathways because the natural products they produce are especially relevant to the pharmaceutical industry. We aimed to develop a tool that selectively labels certain carrier protein-dependent pathways such as fatty acid synthase (FAS), polyketide synthase (PKS), and nonribosomal peptide synthetase (NRPS) pathways. To address this concept, we synthesized a fluorescent fatty acid synthase targeting probe, 5-(4″-dimethylaminophenyl)-2-(4′-phenyl)oxazole (dapoxyl), which was utilized and exhibits a change of emitted wavelength and intensity based on its chemical environment, also known as solvatochromism. Solvatochromism was exhibited when the probe was loaded onto a carrier protein independently and with partner proteins, but the structural data to explain the mechanism is lacking. X-ray crystallography was the leading technique to obtain structural insight into the carrier protein and its probe loading mechanism. Multiple iterations loading the dapoxyl probe onto a carrier protein and its partner protein were tested to maximize labeling and crystallization efficiency for crystallization efforts.

Analyzing protein-protein interactions between the enoyl and keto reductases, InhA and MabA, with the carrier protein, AcpM, from Mycobacterium tuberculosis

Analisa Romberg, Samantha Parker, **Talisa Perez, Desirae A. Mellor**

Principal Investigator: Dr. Michael D. Burkart

Mycobacterium tuberculosis (Mtb) is a widespread respiratory pathogen that kills millions every year. While antibiotics such as isoniazid can be effective, treatment is challenged by the development of antibiotic resistance and Mtb's unique cell wall. The cell wall includes mycolic acids, which are long fatty acid chains produced by the type-II fatty acid biosynthesis pathway (FAS-II) where the central carrier protein, AcpM, is involved in specific protein-protein interactions (PPIs). In FAS-II, AcpM must have these specific interactions with multiple enzymes to turn malonyl-CoA into the fatty acid that will become a mycolic acid, though the many nuances of this pathway are still largely unknown.

To study these protein-protein interactions, we can use methods that trap the proteins in chemically relevant bound complexes, allowing for a crystal structure to be grown and analyzed. In Mtb, the enoyl reductase, InhA, reduces the double bond in enoyl-AcpM to create acyl-AcpM. The ketoreductase, MabA, reduces the ketone in the β-ketoacyl-AcpM to an alcohol to form β-hydroxyacyl-AcpM. Here we report our methods for the generation and purification of InhA and MabA and their applications towards crosslinking with AcpM. The generation and purification of InhA and MabA allow further analysis of the FAS-II pathway, giving more insight into potential drug targets for rational drug design.

<u>Investigating the Role of Heparan Sulfate in Regulating FGF1 Signaling in Adipose Tissue</u>

***Saurabi Sakthivel*, Chung-Jui Yu**
Principal Investigator: Dr. Philip L. S. M. Gordts

Fibroblast growth factor 1 (FGF1) is a critical regulator of adipose tissue metabolism and remodeling, known to suppress lipolysis and lower blood glucose levels in insulin-resistant states. Effective FGF1 signaling requires heparan sulfate (HS) as a co-receptor to stabilize the FGF1–FGFR1 complex, facilitating receptor binding and downstream activation. HS is a complex, highly sulfated carbohydrate attached to a distinct set of proteins found on the surface of nearly all animal cells and within the extracellular matrix. It interacts with a wide range of proteins to regulate key biological processes like growth factor signaling, inflammation, and blood coagulation. Modifications to the structure and sulfation pattern of HS can significantly impact the efficiency of growth factor signaling, including FGF1 signaling.

To investigate the role of adipose tissue HS in metabolic FGF1 signaling, we conducted in vitro and in vivo experiments using 3T3-L1-derived adipocytes and high-fat diet (HFD) mouse models. FGF1-induced activation of the MAPK/ERK pathway was assessed by measuring phosphorylated ERK (pERK) as a readout of metabolic signaling strength. Our results demonstrate that cell surface HS is critical to the concentration- and time-dependent increase in pERK activation, implicating HS as a central regulator for FGF1 signaling in adipocytes.

These findings underscore the importance of HS in regulating FGF1 activity in adipose tissue and suggest that modulating HS composition may offer a novel avenue for improving metabolic health. Future studies will further explore the downstream metabolic consequences of altered HS–FGF1 interactions in vivo.

uPAR is a major effector of p53 gain-of-function mutations in gemcitabine-treated pancreatic ductal adenocarcinoma

***Parnian Sartip*, Carlotta Zampieri**
Principal Investigator: Dr. Steven L. Gonias

In pancreatic ductal adenocarcinoma (PDAC), chemotherapy may select for cancer cells with increased capacity for invasion and metastasis. The responsible mechanisms remain incompletely elucidated; however, TP53, which carries gain-of-function (GoF) mutations, has been implicated. PLAUR encodes a cellular receptor, uPAR, which promotes cancer invasion, metastasis, epithelial-mesenchymal transition (EMT), and resistance to chemotherapy. Mining TCGA showed that in PDAC, PLAUR expression is substantially increased and the extent of increase correlates with cancer stage. High PLAUR expression is associated with decreased overall, disease-free, and progression-free survival. PLAUR expression is increased in PDACs with TP53 GoF mutations. In PANC1 and MIA PaCa-2 PDAC cells, which express TP53 GoF mutations, gemcitabine treatment increased PLAUR expression and ERK1/2 activation in surviving cells. The surviving cells also demonstrated increased migration and invasion. Silencing TP53 or PLAUR blocked the gemcitabine-induced increase in uPAR abundance, ERK1/2 activation, cell migration, and invasion. scRNA-Seq of human PDACs showed that PLAUR expression is increased in specific clusters of malignant epithelial cells post-treatment. High PLAUR expression was associated with increased basal cell-like gene expression signatures, copy number instability and variation, and EMT signatures. Mutated TP53-induced PLAUR expression represents a major pathway by which PDAC treatment may increase cancer cell aggressiveness.

<u>Using Computational tools to find more optimized drug candidates to treat TB</u>

***Praharshitha Thumati*, Haixin Wei**
Principal Investigator: Dr. J. Andrew McCammon

Mycobacterium tuberculosis (Mtb), the bacteria that causes tuberculosis (TB), is estimated to affect a quarter of the world's population and kills more than a million people per year. The current treatments for TB can take six months or longer. Computational tools can optimize and discover drugs, which can lead to a more efficient and faster cure. Cytochrome bd, which is an oxidase found in the electron transport chain (ETC) of Mtb, is a drug target that will be the focus of this project. Previous research on this project has discovered three drug candidates that inhibit the function of cytochrome bd, which would in effect kill the bacteria. Over the summer, I will be running Molecular Dynamic (MD) simulations on the new drug candidates that were tested experimentally by our collaborators. From these MD simulations, we can determine the potential of the various ligands as TB drugs, optimize the binding by adding customized top-hit fragments, and potentially find a better cure for TB.

<u>Mechanistic analysis of cortex detachment and scramblase activity in cellular blebbing</u>

Sivani Vedula, Rachel Rosal, **Adrian Wong**
Principal Investigator: Dr. Itay Budin

Cells are routinely exposed to mechanical stressors that demand rapid membrane adaptation to a changing environment. One such cellular response is plasma membrane blebbing which involves the formation of membrane protrusions due to increased cytosolic pressure. A sudden elevation of intracellular calcium triggers detachment of the actin cortex and activation of the scramblase enzyme TMEM16F that disrupts the asymmetric distribution of lipids between leaflets. This leads to the exposure of phosphatidylserine on the outer leaflet and promotes bleb formation. While both cortex detachment and scramblase activity are known drivers of membrane blebbing, the mechanistic relationship between them remains unclear.

Our lab investigated the connection between cortex detachment and membrane scrambling, utilizing live-cell imaging to track their dynamics. We found that scrambling occurs at discrete membrane regions gated by cortex detachment. To probe this further, we developed biosynthetic tools enabling photo-optic manipulation of the cortex to directly test its influence on TMEM16F. We also analyzed the biophysical constraints on phosphatidylserine diffusion using fluorescence recovery after photobleaching (FRAP) with leaflet specific probes.

Our findings support a model in which scrambling is restricted to cortex-detached regions, and barriers within the plasma membrane restrict phosphatidylserine diffusion from locally scrambled regions. This work enhances our lab's understanding of how cortex mechanics regulate membrane scrambling and bleb formation, while introducing new tools and quantitative measurements to study these processes with greater precision.

<u>eIF5A controls mitoprotein import by relieving ribosome stalling at TIM50 translocase mRNA</u>

Madeleine E. Volfbeyn, **Marina Barba-Aliaga**
Principal Investigator: Dr. Brian M. Zid

Efficient import of nuclear-encoded proteins into mitochondria is crucial for proper mitochondrial function. The conserved translation factor eIF5A binds ribosomes, alleviating stalling at polyproline-encoding sequences. eIF5A impacts mitochondrial function across species, though the precise molecular mechanism is unclear. We found that eIF5A depletion in yeast reduces the translation and levels of the TCA cycle and oxidative phosphorylation proteins. Loss of eIF5A causes mitoprotein precursors to accumulate in the cytosol and triggers a mitochondrial import stress response. We identify an essential polyproline protein as a direct target of eIF5A: the mitochondrial inner membrane protein and translocase component Tim50. Thus, eIF5A controls mitochondrial protein import by alleviating ribosome stalling along Tim50 mRNA at the mitochondrial surface. Removal of polyprolines from Tim50 partially rescues the mitochondrial import stress response and translation of oxidative phosphorylation genes. Overall, our findings elucidate how eIF5A impacts the mitochondrial function by promoting efficient translation and reducing ribosome stalling of co-translationally imported proteins, thereby positively impacting the mitochondrial import process.

Tethered by Similarity, Defined by Difference: Dissecting VAPA and VAPB Function Across Species

***Arisara Weeranarawat,* Safal Shrestha**
Principal Investigator: Dr. Christopher Obara

Membrane contact sites (MCSs) are essential and ubiquitous structures that facilitate intra-organelle and cellular signaling. Identifying and characterizing the proteins involved in MCSs provides critical insights into how cells regulate inter-organelle communication. Vesicle-associated membrane protein-associated proteins (VAPs), particularly the paralogs VAPA and VAPB, are ER membrane proteins that serve as tethers at contact sites between the ER and various organelles, including mitochondria and endosomes. These proteins play crucial roles in cellular processes such as lipid transport and neurotransmitter release. Notably, mutations in VAPB have been linked to neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). Although VAPA and VAPB share approximately 83% sequence similarity, the conservation of specific domains and the evolutionary divergence of their functions across orthologs remain unclear.

To address this, we performed multiple sequence alignments (MSA) and structural alignments of orthologous VAPA and VAPB sequences across multiple organisms. Our results reveal that the major sperm protein (MSP) domain is highly conserved between the two paralogs, exhibiting 82% sequence identity across species. In contrast, the coiled-coil and transmembrane domains show greater divergence, with 56% and 25% sequence identity, respectively. Additionally, we observed significant differences in the two loop regions connecting the three domains, both in sequence length and diversity, suggesting these regions may be key mediators of functional specificity. Ongoing molecular dynamics simulations aim to further elucidate how these sequence differences modulate the structural dynamics of VAP proteins, ultimately contributing to their distinct functional roles.

Organic Division

Alvarez, Paolo

Aravamuthan, Mira

Azizyan, Narek

Chang, Erin

Frisch, Thomas

Patel, Aarya

Tsubouchi, Emiri

Yap, Donnalie

<u>Cyclic Bent Allenes as Catalytic Single Electron Super-reductants</u>

***Paolo Alvarez,* Patrick Yorkgitis**
Principal Investigator: Dr. Guy Bertrand

Since their discovery, stable singlet carbenes have found myriad applications in catalysis, mainly as ligands for transition metals but also as organocatalysts. Notably, while carbene-based organocatalysis has been long known, such transformations have been restricted to ionic, two-electron processes. Recently, our group demonstrated that carbenes can function as catalytic reducing agents via single electron transfer (SET) pathways. Existing single electron reductants are either metallic, reliant on photocatalysts, or used stoichiometrically. In contrast, carbenes are organic species and they are less toxic and less expensive than their organometallic counterparts.

To expand the scope of catalytic carbene-mediated SET chemistry, we propose the development of very electron rich carbenes, namely cyclic bent allenes (CBAs), as single electron super-reductants. To achieve this, we will synthesize novel CBAs bearing carbon-based substituents. Known free CBAs feature heteroatom-based substituents, which our group recently found to enable decomposition pathways wherein these substituents function as leaving groups. C-C bonds are harder to break and carbon-based substituents such as aryl groups are not leaving groups, so the CBAs we synthesize should be more robust than the known heteroatom-substituted CBAs. With new CBAs in hand, we will apply them to the reduction of synthetically valuable but poorly oxidizing substrates such as haloarenes.

CBAs may replace existing metal-based catalysts that are toxic and expensive. Additionally, the extraction and purification of precious metals are responsible for 8% of global energy consumption and 10% of global emissions.

<u>Efforts Toward Self-Assembling Aramid Amphiphiles Containing N-Heterocyclic Carbene Pentafluorophosphorous Adducts</u>

Mira Aravamuthan, **Rylan Rowsey**
Principal Investigator: Dr. Nathan Romero

N-heterocyclic carbene pentafluorophosphorous (NHC-PF5) adducts are highly fluorinated, zwitterionic compounds with high thermal resilience, hydrolytic stability, and are air and moisture-stable. Our lab previously developed a mild and operationally simple synthetic route to synthesizing these adducts. This facilitates incorporating NHC-PF5 functionality into new materials with potential applications as biomedical imaging agents and anti-fouling surface coatings. To generate NHC-PF5-containing materials with microstructural features suitable for biomedical applications, we sought to exploit a molecular architecture established by the Ortony Lab at UC San Diego, which developed a family of Kevlar-inspired aramid amphiphiles that form air-stable nanoribbons via spontaneous self-assembly in water.

The goal of this research is to synthesize and characterize self-assembly in aramid amphiphiles incorporating NHC-PF5 adducts as the hydrophobic tail group. To this end, we have developed a mild, simple, and scalable synthetic route using iterative amide couplings that is modular with respect to the hydrophobic, NHC-PF5 tail group. This route allows for various NHC-PF5 adducts and hydrophilic head groups to be attached, enabling characterization of self-assembly across a novel series of NHC-PF5-containing aramid amphiphiles.

<u>Targeting the Plasmodium falciparum proteasome with optimized KZR-616 analogs</u>

Narek Azizyan, Chandler Huang, **Jehad Almaliti**
Principal Investigator: Dr. William Gerwick

The Plasmodium genus is responsible for malaria, a disease which kills over half a million people a year, the majority of which being children under 5 years old. This widespread infection is due, in part, to the parasite's resistance to conventional anti-malarial drugs. Plasmodium falciparum is the most violent malaria-causing species of this genus. Epoxyketone-based proteasome inhibitors, from the human immunoproteasome inhibitor zetomipzomib (KZR-616), which is currently in clinical trials, are being explored as a potentially effective and selective treatment against P. falciparum by targeting its proteasome's β5 subunit. Here, synthetic methods for these compounds and future plans for this project will be discussed.

<u>Improving Membrane Permeability of Proteolysis Targeting Chimeras through Prodrugs</u>

***Erin Chang*, Georges Leconte**
Principal Investigator: Dr. Fleur Ferguson

Proteolysis targeting chimeras (PROTACs) are heterobifunctional molecules which catalytically degrade a protein of interest (POI) via hijacking of endogenous cellular protein clearance mechanisms. Most PROTACs consists of 3 main parts: a POI warhead, an E3 ligase ligand, and a linker to connect the two. This conserved structure imparts a modularity enabling rapid synthesis of a variety of PROTACs, although at the consequence of poor pharmacokinetic properties, including an increased molecular weight. This characteristic serves as a significant impediment to membrane permeability, especially true for targets in the central nervous system (CNS) behind the blood brain barrier (BBB), the tight junction of endothelial cells in CNS capillaries that prevents entry of a reported 99% of all small molecules. While compound optimization is possible, it is highly resource intensive, creating a bottleneck in the synthesis of CNS PROTACs. One approach to this problem is a prodrug strategy, in which the parent drug is modified with a labile promoiety, which detaches in situ, to improve pharmacokinetic factors. This project aims to develop and apply a prodrug strategy for rapid improvement of PROTAC pharmacokinetic properties by conjugating lipid promoieties to the key interacting site on the conserved von-Hippel Lindau (VHL) recruiting ligand, thus improving the lipophilicity and subsequent BBB permeability. By working with, as opposed to against, the modular nature of PROTACs, this work aims to apply and develop an accelerated and generalizable approach to synthesize PROTAC prodrugs for more rapid entry of degraders into CNS space.

<u>Renewable and Biodegradable Polyurethanes as Alternatives for Commercial Plastics</u>

Thomas Frisch, **Caitlin Hudecek, Aaron Bruckbauer**
Principal Investigator: Dr. Michael Burkart

The devastating environmental consequences of plastic production and waste are undeniable. Each year, over half a billion tons of plastic are produced from petroleum-sourced materials. On every continent and in every ocean, plastic pollution destroys ecosystems. Sustainable alternatives for current practices in the plastic industry are needed. Polyurethanes, which account for 10% of global plastic production, are a versatile class of plastic made up of two major components. Variation in these components, polyol and diisocyanate, have a dramatic effect on the properties of polyurethanes. The vast majority of polyurethanes are produced with the use of aromatic diisocyanates, which have not been shown to be renewably sourced. In this project, polyester polyols composed of synthetic algae-based monomers were utilized to produce 100% renewable and biodegradable polyurethanes with aliphatic diisocyanates. In subsequent studies of aromatic and aliphatic components of polyurethanes, high performance sustainable alternatives were developed for a wide range of polyurethane applications. These alternatives display mechanical and thermal properties that rival commercial standards, along with excellent rates of biodegradation. This work suggests that commercial polyurethanes can be reengineered for renewability and biodegradability.

<u>Isolation of secondary carbon centered radicals</u>

Aarya Patel, **Levan Gojiashvili**
Principal Investigator: Dr. Guy Bertrand

Organic paramagnetic species play a vital role in many chemical reactions, which have found various applications in synthetic, polymer, and medicinal chemistry. As of today, most of the isolable radicals are heteroatom centered. In the carbon series, a few tertiary carbon centered radicals are isolable, but secondary and primary carbon radicals can only be observed spectroscopically. Characterization of isolated carbon centered radicals will significantly facilitate mechanistic studies and might find new applications.

Previously, our group reported the successful isolation of a crystalline pentadienyl-type radical featuring a central secondary carbon. This radical was obtained via single-electron reduction of a bis(imino)carbene conjugate acid. Its stability is attributed to the two N-heterocyclic imine groups, which provide both electronic stabilization and steric protection. Density functional theory calculations indicated that the central secondary carbon serves as the primary spin carrier. In this work, we are substituting one N-heterocyclic imine by a smaller R group to increase the reactivity of the secondary radical, while maintaining electron delocalization. By using 2,4,6-trimethoxyphenyl as a substituent, the corresponding conjugate acid is obtained, and single electron reduction is performed. Electron paramagnetic resonance spectroscopy (EPR) and Proton nuclear magnetic resonance (1H NMR) confirmed the presence of a radical which is in equilibrium with its dimer counterpart. Based on these results, the effect of different substituents will be investigated, and the reactivity of these species will be studied.

<u>Synthesis of Tight-binding Probes: Binding Enoyl Reductase and Acyl Carrier Protein in Fatty Acid Biosynthesis</u>

Emiri Tsubouchi, **Yuta W. Moriuchi**
Principal Investigator: Dr. Michael D. Burkart

Enoyl Reductase (ER) have been identified as a critical factor for bacterial cell survival, and understanding their protein-protein interactions with Acyl-Carrier Protein (ACP) in fatty acid biosynthesis can provide valuable insights. The project aims to understand the protein-protein interactions between Enoyl Reductase and Acyl-Carrier Protein in fatty acid biosynthesis.

To achieve this, the research follows three key steps: establish a tight binding probe panel to crosslink the proteins, explore the efficiency of the tight binding probes, and understand and characterize the ACP-ER complex.

Currently, the project is focused on the first step: the synthesis of probes with varying chain lengths. By creating and testing probes of different lengths, the structure that best facilitates strong interactions will be determined. The creation of the probe involves a multi-step synthesis requiring careful execution and analysis at each step. Specifically, the process includes setting up reactions by adding chemicals, monitoring the progress, removing byproducts after the reaction is completed, analyzing the structure after each step, and proceeding to the next reaction. These steps are repeated systematically until the final product, the probe, is successfully formed.

Through these synthetic techniques, a set of new probes to study the interactions of the proteins(ER and ACP) will be created.

Ruthenium-Mediated 6pi Cyclization in Conjugated Dienyne Systems: Discovery of Unprecedented Acyl Migration

***Donnalie Yap,* Andrew Chua, Dipendu Mandal**
Principal Investigator: Dr. Joseph O'Connor

Ruthenium catalysts are utilized across all branches of chemistry, from the synthesis of active pharmaceutical ingredients to catalyze ammonia decomposition. Ruthenium catalysts are particularly useful for cyclization and forming new carbon to carbon bonds. However, they are difficult to synthesize due to Ru oxides vaporizing at high temperatures, which are typically needed for catalytic reactions. We developed new synthetic pathways to more efficiently and effectively synthesize ruthenium catalysts. The synthesis of potential ligands for ruthenium catalysts was initiated with cyclohexanone, which underwent a Wilsmeir-Haack reaction by using dimethylformamide (DMF) and phosphorus tribromide (PBr3) in chloroform to yield a brominated cyclohexene derivative. This derivative was obtained as an oil, which then participated in a Sonogashira coupling reaction. This sp involved the use of ethynyltrimethylsilane (TMS-acetylene) in the presence of a palladium catalyst [Bis(triphenylphosphine)palladium dichloride, Pd(PPh3)2Cl2] and copper (I) iodide (CuI) in triethylamine (Et3N) to introduce a trimethylsilyl-protected carbaldehyde, or 2-((trimethylsilyl)ethynyl)cyclohex-1-ene-1-carbaldehyde. This was then isolated and underwent a Corey Fuchs reaction to create a di-brominated alkyne product, which then underwent numerous deprotection reactions (isopentyl and TMS) with THF as the solvent. This created a diene-yne product that was then methylated with LHMDS and iodomethane, adding an ether group to the molecule. Lastly, ruthenium cyclopentane and pentamethylcyclopentadiene were cyclized with RuCp and RuCp*, noting a full consumption of the starting material and yield of a brown solid, producing the desired ruthenium catalyst.

Physical/Analytical Division

Albert, Catherine

Rhoads, Alison

Solomon, Zoe

Ventura, Xitlali A.

Wang, Justin

Wolek, Liwia

Wu, Kyra

<u>Solid Phase Extraction of PFAS Compounds</u>

Catherine Albert, Rachel Fishman, Elizabeth Pogue, Sherly Ochoa-Morin, Andrea Armendi, Angel Salas, Jagger Nelson, Jose Antonio Puron Guerrero, Lisa Jones
Principal Investigator: Dr. Robert Pomeroy

The bioaccumulation of PFAS in the environment requires crucial examination. California regulations will increasingly require testing of these molecules with high precision. PFAS have high thermal stability, are resistant to degradation, and have surfactant properties, allowing them to bioaccumulate and be very persistent in the environment. In an attempt to confirm the efficiency and validity of the methodology for their detection, PFAS analysis was conducted on parallel extractions of aqueous, soil, and tissue samples dosed with known concentrations.

Samples were analyzed using Solid Phase Extraction techniques. The samples were then injected into the Vanquish UHPLC coupled with an Orbitrap MS. The resulting chromatograms were compared, and the peak areas and heights were used to quantify the PFAS. Initial results show that PFAS recovery varied by matrix and sample concentration.

Overall, PFAS recovery varied by sample type and spike level, with tissue samples showing the most robust recovery and the greatest consistency. Ongoing analysis of recovery trends will focus on identifying possible sources of variability. These findings will inform recommendations for improving extraction protocols and method reproducibility across complex matrices.

Data-Driven Many-Body Framework for Organic Molecules

***Alison Rhoads*, Ruihan Zhou**
Principal Investigator: Dr. Francesco Paesani

Data-driven many-body energy (MB-nrg) potential energy functions (PEFs) provide predictive molecular models for large systems with quantum mechanical accuracy, positioning them as a powerful tool in investigating structural, thermodynamic, dynamical, and spectroscopical properties of generic molecular systems from the gas to the condensed phase.

We have developed the first MB-nrg PEF for a ring-molecule, phloroglucinol (1,3,5-trihydroxy benzene) dihydrate, a highly effective organic ice nucleator. A thorough understanding of phloroglucinol's ice-binding mechanism would allow us to harness its natural properties to promote ice crystallization through applications like weather engineering, and more efficient thermal storage techniques. We show that our MB-nrg PEFs describe the energetics of isolated phloroglucinol molecules and their two-body interactions with high accuracy, providing insights into phloroglucinol crystal formation and stability. We also present results for the interaction of phloroglucinol with water; identifying valid conformers of phloroglucinol dihydrate and simulating its behavior in water represent the first step toward developing a comprehensive description of ice-binding molecules' interaction with water, their ice-binding sites, and the kinetics of ice nucleation and antifreeze behavior.

<u>Toward Data-Driven Many-Body Simulations of Biomolecules with Chemical Accuracy</u>

***Zoe Solomon*, Richa Rashmi, Ruihan Zhou**
Principal Investigator: Dr. Francesco Paesani

Accurate molecular models are a critical aspect of computational biochemistry, as they enable us to predict behavior and chemical properties of complex systems. As part of an effort to model generic biomolecular systems, we introduce a data-driven many-body energy (MB-nrg) potential energy function (PEF) to serve as a predictive molecular model of the simplest amino acid, glycine, which is an essential building block in proteins and peptides. These characteristics of glycine make it an ideal starting point as a transferable model that can be applied to more complex systems in the future. To provide direct comparisons with high-resolution vibrational spectra measured experimentally, we focus on the protonated version of glycine. Analyses of structural properties and vibrational spectra of protonated glycine in both gas-phase water clusters and liquid water indicate that our MB-nrg PEF provide a realistic description of the hydration structure, enabling a detailed characterization of the hydration process, one water molecule at a time. Our study represents a first step toward the application of MB-nrg PEFs to entire proteins, which will thus enable the modeling of generic biomolecules with quantum-mechanical accuracy.

Field study analysis, quantification, and impacts of ionic species in coastal aerosols using positive ion chromatography

Xitlali A. Ventura, **Amelie Creekmore, Kanuri Roundtree**
Principal Investigator: Dr. Jonathan H. Slade

When ocean waves break, sea spray aerosols (SSA) are created. SSA affects the climate and environment, as they make up a significant source of aerosol mass in the atmosphere. A field study was conducted in the winter and summer of 2023 to determine seasonal and diurnal variations of components, including emerging contaminants, in ocean water and SSA. SSA was collected on filters, and the concentrations of essential ions, including sodium, magnesium, and calcium, were measured in the air. These ions and other chemical species were compared to their concentrations in ocean water. Sodium ion concentrations were measured to trace the presence of SSA and to determine the enrichment of different components in SSA. In addition, measurements of particle bounce (i.e., phase state) were conducted to better understand the chemical drivers of SSA phase state. Due to their strong ionic bonds with organic material in SSA, divalent cations, like calcium and magnesium, tend to promote more viscous phase states. This poster will report ions' concentrations and extraction efficiencies in SSA from the filter samples, analyzed via ion chromatography (IC). The ion concentrations will be used to calculate SSA enrichment factors of organic chemical contaminants, like ultraviolet filters, and to better understand the role of divalent cations in modulating SSA phase states. Overall, the results seek to improve our understanding of chemical and ionic interactions in SSA and, thus, how SSA impacts atmospheric chemistry.

<u>Esters on the Fly: A Catalyst-Free Synthesis via Atomization</u>

Justin Wang, **Lincoln Mehndiratta, Jonathan Slade**
Principal Investigator: Dr. Vicki Grassian

Esterification is a critically important synthesis reaction with a broad range of applications. Typical bulk esterification reactions require a strong acid catalyst and/or heat in order to overcome the activation energy of the reaction. We developed a new green synthesis of esters without the use of catalyst or heat via atomization of bulk solutions. In separate experiments, solutions of alcohols in high excess (methanol, ethanol, and isopropanol) combined with organic acids (C_8, C_9, C_{12}, and C_{16}) were aerosolized using a TSI atomizer for aerosols ranging in size from 1 to 20 μm. Following evaporation of the alcohol, the residual was then analyzed using different techniques including Optical Photothermal Infrared (O-PTIR) spectroscopy and mass spectrometry. For O-PTIR analysis, the ester and any remaining acid could be detected. Remarkably, for smaller acids like octanoic (C_8) and nonanoic (C_9) acids, the reaction achieved near-quantitative ester yields. In contrast, larger acids such as lauric (C_{12}) and palmitic (C_{16}) acids yielded esters at approximately 30%. Moreover, the presence of water in the initial solution significantly inhibited esterification, underscoring the importance of non-equilibrium kinetics in this process. Overall, these results highlight aerosolization as a highly efficient, environmentally friendly pathway for ester synthesis, demonstrating significant potential for advancing green chemistry by eliminating the need for heat or catalysts.

p-Azaquinodimethanes for Triplet-Triplet Annihilation Upconversion

***Liwia Wolek,* Lukas Naimovicius**
Principal Investigator: Dr. Andrew Pun

Triplet-triplet annihilation (TTA) upconversion (UC) is a process that converts two low-energy photons into one higher-energy photon. It is a bimolecular process involving a sensitizer and an annihilator. The sensitizer absorbs low-energy light, while the annihilator emits high-energy photons from its excited single state upon TTA. TTA-UC can demonstrate high efficiency upon incoherent light excitation, like sunlight or LEDs, which highlights its potential for diverse applications including solar energy, biological imaging and photocatalytic processes. While sensitizers for TTA-UC have been investigated, the development of annihilator molecules remains limited, thereby motivating focused research efforts towards the design of high-performance annihilators. In this work, we expand the library of novel annihilators, exploring p-azaquinodimethanes (AQMs) for TTA-UC use. We introduce AQMs, unexplored family of biradicaloid molecules that can function as annihilators. AQMs are known to be weakly emissive; however, we have shown that they have a capability to upconvert in both solution and solid-state systems. The results demonstrated that AQM-based annihilators are not only effective in TTA-UC but are also highly tunable via chemical synthesis, enabling modulation of their properties for diverse applications.

Host-directed, bioelectronic immunomodulation for protection against emerging pathogens

Kyra Wu
Principal Investigator: Dr. Allison Yorita

Historically, the treatment of acute, life-threatening infections caused by emerging pathogens has been hindered by delays in the development of pathogen-specific therapies. This lag, especially when coupled with immune system dysfunction, contributes to increased mortality and a higher incidence of critical illness—placing significant strain on healthcare systems. Cytokines are valuable systemic biomarkers of immune activity, but current quantification methods are resource-intensive and lack real-time capabilities. Bioelectronic stimulation of the vagus nerve has shown promise in modulating immune responses, though its use has largely been limited to chronic autoimmune conditions. To address this gap, we are developing a minimally invasive system that enables real-time cytokine detection and immune modulation via vagus nerve stimulation (VNS), offering a novel approach to treating acute, critical illness caused by pathogen infection.

Acknowledgements

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We are grateful for the time, expertise, and encouragement from our visiting judge associated with Catalent, and our faculty, postdoc, and graduate student judges from the UC San Diego Department of Chemistry and Biochemistry. ACS-SA would also like to acknowledge the support from the Department of Chemistry and Biochemistry, the Dean's Office, the Division of Physical Sciences, and the Parent's Fund. We are beyond honored to have been supported by so many people in our mission to provide opportunities to the UC San Diego Department of Chemistry and Biochemistry.