Poster abstract listed

Poster number: 1
Assignment: Session 1 (odd number posters) 11-12 pm
Name: Phillip S. Rushton
Title: Resistance Fighters: Developing a Novel HMG-CoA Reductase Inhibitor to Combat Gram-Positive Bacteria
Authors: Phillip S. Rushton, Nic Steussy, Sucharita Bose, Daneli Lopez-Perez, Tim Schmidt, Mohamed Seleem, Mark Lipton and Cynthia Stauffacher
Abstract: Multi-drug resistant bacteria infect millions of people per leading to significant costs for society. With fewer antibiotic drugs in development there is a growing need for drugs that target novel mechanisms. The bacterial mevalonate pathway is essential for pathogenic gram-positive bacteria and possesses distinct elements from evolutionarily higher mevalonate pathways, making it an ideal target for drugs. We have screened thousands of compounds against HMG-CoA reductase (HMGR), the rate limiting enzyme of the mevalonate pathway, leading to the discovery of a low micromolar inhibitor. Guided by enzyme kinetics and structures of HMGR bound with lead compounds it was possible to synthesize better inhibitors. In vivo anti-bacterial experiments revealed that these compounds can inhibit bacterial growth of drug-resistant bacteria. Recent work aims to expand the design parameters of the lead compound and/or generate new lead compounds by discovering smaller inhibitory chemical fragments that can be synthetically combined. A library of chemical fragments was screened against E. faecalis and S. aureus HMGR for enzyme inhibition. The best compounds were soaked into the more robust Pseudomonas mevalonii HMGR crystals to evaluate their binding site. Informed by these structures, a more effective antibiotic can be generated with lower inhibitory constants and effective bacterial control.

Poster number: 2
Assignment: Session 2 (even number posters) 1-2 pm
Name: Laura Chamness
Title: Investigating Synonymous Mutational Effects on Co-translational Protein Folding
Authors: Laura M. Chamness (1), Disha Patel (1), Rohan Bhardwaj (2), Jonathan P. Schlebach (2) & Patricia L. Clark (1)
(1) Department of Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN, 46556 USA
(2) Department of Chemistry, Purdue University, West Lafayette, IN, 47907 USA
Abstract: Many proteins rely on co-translational folding pathways to achieve their native structure. Synonymous mutations are often assumed to have no effect on protein folding because they do not alter the encoded amino acid sequence, but have been shown to perturb protein structure and folding by modulating the rate of translation elongation. Elucidating the mechanisms by which synonymous mutations affect protein folding would enhance our understanding of protein production in the cell. Here, we show that synonymous mutations affect the production of functional fluorescent proteins in E. coli. An eGFP coding sequence mutated to use different patterns synonymous codons led to decreased fluorescence intensity in cells, suggesting that codon usage may affect translation initiation, translation elongation, and/or co-translational folding in a manner that interferes with functional eGFP production. Similarly, synonymous mutations in a secreted moxVenus

protein caused diminished fluorescence intensity in cells. Crucially, the fluorescence reduction observed for common codon versions of eGFP and moxVenus indicate the codon effect is unrelated to issues with translational efficiency. Together, our results suggest that synonymous codon usage can affect co-translational folding and functional protein production in E. coli for both cytosolic and secreted proteins.

Poster number: 3
Assignment: Session 1 (odd number posters) 11-12 pm
Name: Emmanuel Oluwarotimi
Title: Biochemical and Cryo-EM Structural Studies of GTP Cyclohydrolase I from Candida auris: A Potential New Target for Antifungal Therapy
Authors: Emmanuel Oluwarotimi1, Frank Vago, Indranil Arun Mukherjee, Andrew Mesecar
Abstract: Fungal infections cause approximately 1.6 million deaths annually, with Candida species as being the principal causative agent (WHO report. The rise in antifungal resistance highlights the need for novel antifungal drug targets. Guanosine triphosphate cyclohydrolase I (GCH1), essential in the tetrahydrofolate biosynthesis pathway, is a promising candidate due to its critical role in fungal DNA replication and cell survival. Notably, human GCH1 is regulated by GCH1 feedback regulatory protein (GFRP), a protein absent in Candida, suggesting a potential for developing selective inhibitors. In this study, we characterized Candida GCH1 (cGCH1) using biochemical, biophysical, and structural methods. Full-length cGCH1 was expressed in E. coli and purified, yielding 11 mg/L of culture. Kinetic assays revealed a kcat of 0.5 ± 0.02 min^{-1} and KM of 100 ± 16 μM, with a catalytic efficiency of 5,000 ± 825 $M^{-1}min^{-1}$, comparable to GCH1 from other E. coli and humans. Thermal stability (Tm) was 74.9 ± 0.4°C, and mass photometry confirmed a decameric assembly. Cryo-EM revealed a D5-symmetric homo-decamer with anisotropic 3.2 Å resolution due to orientation bias. Efforts are ongoing to optimize cryo-EM sample preparation and obtain isotropic maps. Future work includes inhibitor screening to evaluate cGCH1 as a selective antifungal drug target.

Poster number: 4
Assignment: Session 2 (even number posters) 1-2 pm
Name: Faith McCauley
Title: Investigating the role of the X-Y linker and Y-box in phospholipase Ce (PLCe) regulation
Authors: Faith McCauley, Elisabeth Garland-Kuntz, Vaani Ohri, Angeline Lyon
Abstract: Phospholipase Ce (PLCe) plays a crucial role in protecting the heart from oxidative stress. PLCe is the largest member of the PLC superfamily and shares the conserved core architecture. PLCe is regulated by N- and C-terminal domains that flank the core, along with two insertions within its catalytic TIM barrel domain. The X-Y linker is autoinhibitory and found in most PLC families, blocking the substrate-binding site. In PLCe, we previously identified an amphipathic helix within the linker that is needed for lipase activity. The Y-box is more dynamic than the X-Y linker and only found in the TIM barrel of PLCe. It was previously demonstrated that the Y-box is required for basal and RhoA-dependent activity. How these two elements contribute to PLCe regulation is not known. I hypothesize that the X-Y linker and Y-box regulate lipase activity by optimizing interactions between the catalytic domain and its lipid substrates. I will be using cell-based assays and confocal microscopy to identify residues in these insertions important for activity and colocalization. I will also use cryo-electron microscopy to identify changes in lipase structure

in PLCe variants and in-vitro assays to investigate these findings, test stability, and protein-membrane interactions

Poster number: 5
Assignment: Session 1 (odd number posters) 11-12 pm
Name: Omar M. Qassem
Title: Mechanisms of isoform-specific regulation of adenylyl cyclase 5 by Gαi
Authors: Omar M. Qassem, Indranil A. Mukherjee, Val J. Watts, Carmen S. Dessauer, John J.G. Tesmer
Abstract: Adenylyl cyclases (ACs) are enzymes that catalyze the conversion of ATP into cAMP, a secondary messenger involved in signal transduction downstream of G protein-coupled receptors. The nine AC membrane isoforms are activated by Gαs, and the mechanisms underlying this activation are well understood. However, these isoforms exhibit different responses to Gαi and Gβγ. AC5 is highly expressed in the heart and is involved in cardiovascular disease. It is activated by Gαs and Gβγ, while it is inhibited by Gαi. Our long-term goal is to elucidate the unique regulatory mechanisms of AC5 by different G protein subunits, to develop isoform-selective inhibitors. Recently, a study by our group has reported the cryo-EM reconstitution of AC5 in two states: a complex with Gβγ and a dimer. Gβγ can only bind to a monomer, so it is hypothesized that AC5 is present in an equilibrium between a monomeric and a dimeric state, where Gβγ stabilizes the monomeric one. However, there is no structural information of any AC isoform in complex with Gαi, so how the Gαi fits into this model is still under investigation. In this current study, a preliminary cryo-EM structure of asymmetric AC5 homodimer in complex with Gαi in GDN micelles is produced. According to it, a suggested mechanism for at least part of Gαi regulation is to stabilize a dimeric state of AC5 that blocks Gαs from binding.

Poster number: 6
Assignment: Session 2 (even number posters) 1-2 pm
Name: Grigorii Rudakov
Title: From Design to Gene Suppression - DNA Tetrahedra for In Vitro Therapeutic miRNA Delivery
Authors: Grigorii Aleksandrovich Rudakov, Chengde Mao, Gregory Thomas Knipp, Leopold Noel Green, Tamara Lea Kinzer-Ursem
Abstract: One of the challenges posed by miRNA delivery is transfusing the oligos into the cells. Nanomaterials have been proven to decorate cells efficiently via DNA and protein aptamers, increasing the local drug concentration at the cellular membrane. A novel method of delivering miRNA via DNA-cholesterol anchoring improved the delivery efficiency in vitro in HeLa and HAT pathogenic cells. Here, we report a comparison of in vitro miRNA delivery efficiency and TEG-cholesterol anchoring efficiency of a novel DNA tetrahedra design. The cell lines tested were HEK293 human kidney and CA1A breast cancer cells. We measured the therapeutic effect of delivered miRNA in CA1A by silencing dTomato fluorescent protein. As a result of this work, we present the new DNA tetrahedra design capable of achieving increased delivery efficiency and therapeutic effect.

Poster number: 7
Assignment: Session 1 (odd number posters) 11-12 pm
Name: Cara Trench
Title: Regulation of PLCb2 in Platelet Function

Authors: Cara Trench, Leon Laskowski, Isaac Fisher, Faith McCauley, Angeline Lyon
Abstract: Coronary heart disease (CHD) is the leading cause of death in the United States and worldwide. This disease most commonly develops through the build-up of atherosclerotic plaque in the coronary artery. The phospholipase C (PLC) beta subfamily has been implicated in the buildup of atherosclerotic plaque. These enzymes cleave phosphatidylinositol-4,5-biphosphate (PIP2) at the inner leaflet of the plasma membrane, producing diacylglycerol (DAG) and inositol-1,4,5- triphosphate (IP3). IP3 stimulates Ca2+ release from intracellular storage, which combined with membrane-associated DAG, activates protein kinase C (PKC). Of the PLCβ subfamily, PLCβ2 is of special interest because it is highly expressed in hematopoietic cells and is most sensitive to activation by the Gβγ heterodimer in response to stimulation of Gi-coupled receptors. Gβγ-dependent activation of PLCβ2 leads to platelet aggregation and increased proliferative signaling. Recent studies have shown that PLCβ2 expression is regulated, at least in part by the transcription factor NF-kB. NF-kB is well known for its roles in immune and inflammatory signaling, and thus may be responsible for the increased expression and activity of PLCβ2 in atherosclerosis and inflammation. The objectives of this study are to determine whether NF-kB transcriptional regulation affects the expression and subsequent Gβγ-dependent activation of PLCβ2 to drive CHD.

Poster number: 8
Assignment: Session 2 (even number posters) 1-2 pm
Name: Ryan Rushing
Title: Nuclear Mechanotransduction: Observing Changes in Chromatin Dynamics via dSTORM
Authors: Alexander Q. Phillips, Ryan Rushing, Jing Liu
Abstract: Nuclear mechanotransduction is the study of how forces from outside a cell are transmitted into the nucleus of a cell. The study of these interactions is essential, as many external stimuli on a cell impact gene regulation. Chromatin is the complex that allows DNA to compact into a higher-order structure. The structure and motion of chromatin play a role in how genes are expressed. Our goal is to investigate how external forces impact chromatin dynamics. To do this, we plan to perturb a cell using magnetic tweezers and to observe changes utilizing direct stochastic optical reconstruction microscopy (dSTORM).

Poster number: 9
Assignment: Session 1 (odd number posters) 11-12 pm
Name: Asmita Asmi
Title: Design and Development of Small Molecule Kinase Inhibitors Targeting TAK1 for Inflammatory Diseases
Authors: Asmita R. Asmi, Mandeepkaur Hunjan, Desmond Akwata, Neetu Dayal and Herman O. Sintim
Abstract: Protein kinases regulate cellular functions by transferring the γ-phosphate from ATP to tyrosine, serine, or threonine residues on substrate proteins. This post-translational modification plays a vital role in controlling pathways related to cell growth, differentiation, apoptosis, and immune responses. Dysregulated phosphorylation is associated with numerous diseases, including inflammatory and autoimmune disorders, as well as cancer. Consequently, kinases have become attractive targets for drug development. Despite the existence of over 500 human kinases, fewer than 10% have been successfully targeted by approved drugs.

Transforming growth factor-β-activated kinase 1 (TAK1) is a serine/threonine kinase implicated in several inflammatory and cancer-related pathways. It is activated by proinflammatory cytokines and stimuli such as TNF, IL-1β, LPS, and TGFβ, leading to the activation of downstream signaling cascades including JNK, p38 MAPK, and NF-κB. These pathways are strongly implicated in diseases such as rheumatoid arthritis, inflammatory bowel disease, and pneumoconiosis, where cytokines like IL-1β, IL-6, and TNF are found to be overexpressed and contribute to chronic inflammation and tissue damage.
TAK1 inhibition has shown strong therapeutic promise in preclinical models of inflammatory diseases. Small-molecule TAK1 inhibitors like 5Z-7-oxozeaenol and takinib have effectively reduced inflammation and tissue damage in experimental models. Building on our previous work with morpholino-imidazo[1,2-b]pyridazine TAK1 inhibitors, we developed imidazothiadiazole analogs—structurally distinct five-five fused rings containing sulfur and nitrogen atoms. Compared to the six-five fused ring imidazo[1,2-b]pyridazine containing only nitrogen, imidazothiadiazole features a five-five fused ring with both sulfur and nitrogen. In medicinal chemistry, smaller ring systems often improve bioavailability and selectivity, while sulfur contributes to enhanced bioactivity, metabolic stability, and functional versatility in drug design. The imidazothiadiazole analogs have demonstrated stronger TAK1 inhibition than known compounds like tuspetinib and significantly suppress TNF-α production, a key proinflammatory cytokine.
Our ongoing research aims to evaluate the impact of TAK1 inhibition on IL-6 production and confirm downstream pathway suppression using Western blot analysis. The imidazothiadiazole scaffold offers a versatile platform for the development of additional kinase inhibitors, potentially extending therapeutic applications to oncology and other medical conditions.

Poster number: 10
Assignment: Session 2 (even number posters) 1-2 pm
Name: Shivam Mahapatra
Title: Label-Free Quantification of Apoptosis and Necrosis Using Stimulated Raman Scattering Microscopy
Authors: Shivam Mahapatra, Shreya Shivpuje, Helen Campbell, Boyong Wan, Justin Lomont, Bin Dong, Seohee Ma, Karsten J. Mohn, and Chi Zhang
Abstract: The production of recombinant proteins is fundamental to modern therapeutics and diagnostics, with Chinese hamster ovary (CHO) cells serving as the primary host cells. However, maintaining viable cell populations in bioreactors is challenging, as environmental and chemical stressors frequently trigger apoptosis, disrupting protein manufacturing. Effective, label-free techniques for real-time cell death monitoring are essential for improving production efficiency and product quality. Stimulated Raman scattering (SRS) microscopy is a powerful imaging modality capable of assessing lipid and protein distributions in live cells without the need for exogenous labels. In this study, we utilize SRS microscopy to rapidly and non-invasively distinguish apoptotic and necrotic transitions. Our results indicate that apoptosis corresponds with increased intracellular protein concentration, whereas necrosis is marked by a decline in protein levels. To enhance analytical precision, we developed a machine-learning-driven single-cell analysis pipeline that identifies chemotypic and phenotypic markers of apoptosis and necrosis, enabling the classification of cell subpopulations with distinct stress responses. Additionally, we demonstrate the robustness of this approach across multiple stressors and cell types, reinforcing its broad applicability. This method, when integrated with microfluidic platforms, is expected to facilitate rapid

assessments of cell status and their correlation with CHO cell protein production in inline and online scenarios.

Poster number: 11
Assignment: Session 1 (odd number posters) 11-12 pm
Name: Jessica Prakash
Title: Optimizing Polyplex Delivery of Self-Amplifying mRNA as a Potential Immunotherapy Application for Metastatic Prostate Cancer
Authors: Jessica Prakash
Abstract: Prostate cancer, a leading cause of cancer-related death in American men, frequently metastasizes to bone, significantly reducing survival rates. This study investigates Interleukin-27 (IL-27) delivery using a self-amplifying mRNA (saRNA) vector as a potential immunotherapy for metastatic prostate cancer. While saRNA enables sustained gene expression at lower doses, its large size complicates delivery. Therefore, we hypothesize that a highly cationic reverse nuclear localization signal (rNLSd) polymer will fold saRNA, enhancing membrane penetration and delivery. After formulation, rNLSd polyplexes were characterized using dynamic light scattering, revealing sizes of 50–76 nm and charges of 5-8 mV that suggest high cell permeability with minimal cytotoxicity. To evaluate transfection efficiency, GFP expression was monitored in C2C12 cells over five days using rNLSd-saRNA-GFP polyplexes, mRNA-GFP as a baseline control, Lipofectamine 2000 (L2000) as a positive control, and naked RNAs as a negative control. L2000 exhibited high cytotoxicity despite strong GFP expression, while rNLSd polyplexes maintained better cell viability but low GFP expression. This study suggests rNLSd polyplexes offer lower cytotoxicity than L2000, but further optimization of rNLSd polyplexes is required to enhance gene expression. By overcoming this issue, this research could provide a therapy model for a wide range of cancers and genetic disorders.

Poster number: 12
Assignment: Session 2 (even number posters) 1-2 pm
Name: Douglas Chan
Title: Improving the Bioavailability of Gefitinib Across the Blood-Brain Barrier: A Prodrug Strategy for Inhibition of P-gp-mediated Efflux
Authors: Douglas S. Chan, Christine A. Hrycyna, and Jean Chmielewski
Abstract: The prognosis of non-small cell lung cancer (NSCLC) patients with brain metastases is very poor. Targeted anticancer agents that are used as the first-line treatment of oncogene-driven NSCLC have limited effectiveness in treating brain metastases of mutant NSCLC due to efflux transporters, such as P-glycoprotein (P-gp), that limit drug penetration across the blood-brain barrier (BBB). To address this limitation, we present the development of a dimeric prodrug P-gp inhibitor based on the P-gp substrate and first-line NSCLC targeted therapeutic gefitinib. With the goal of improving gefitinib's therapeutic brain penetration, the gefitinib-based dimer GFTB-Q was designed to have a dual role: to inhibit P-gp efflux at the BBB and to regenerate the monomeric therapy within cellular reducing environments. GFTB-Q exhibited potent inhibition of P-gp-mediated efflux in cell culture, including for human brain endothelial cells. Additionally, reduction of GFTB-Q yielded the regeneration of its monomeric components in vitro and in cells, validating its design as a dimeric prodrug inhibitor of P-gp. Molecular docking was further pursued to provide insight into the prodrug's possible mechanism of inhibition.

Poster number: 13
Assignment: Session 1 (odd number posters) 11-12 pm
Name: Meredith Hansen
Title: The Formidable Dimer Interface of an Emerging Coronavirus and its Challenge for Drug Design
Authors: Meredith Hansen, Uttara Jayashankar, Monika Yadav, Arun K. Ghosh, Andrew D. Mesecar
Abstract: To date, SARS-CoV-2 has caused 7.1 million deaths and 778 million infections worldwide.1 Coronaviruses are classified into 4 genera; beta coronaviruses include SARS-CoV-2 and MERS-CoV; CCoV-HuPn is an alpha coronavirus that infects canines; however, two independent incidences of human infection have been identified4, confirming its status as an emerging zoonotic virus and highlighting its potential to cause a pandemic. Coronaviruses have a single-stranded, a positive-sense RNA genome with two ORFs that encode two polypeptides. 3CLpro, the chymotrypsin-like protease, cleaves the polypeptides into NSPs; this enzyme is essential for replication and well-conserved across genera, making it an ideal drug target.2 Nirmatrelvir is a small-molecule inhibitor approved to treat SARS-CoV-2 infections by inhibiting the 3CLpro enzyme, rendering viral replication impossible. Despite nirmatrelvir's efficacy against SARS-CoV-2, it exhibits limited potency against and induces ligand-induced dimerization in CCoV-HuPn due to the weak dimer interface of its 3CLpro enzyme. Ligand-induced dimerization is a phenomenon in which low inhibitor concentration increases enzyme activity; this has been observed in MERS-CoV3 and increases the complexity of drug design. In this study, we kinetically characterize and perform SAR studies of CCoV 3CLpro to probe the dimer interface. From the insights gained, we strategically modify the Nirmatrelvir scaffold to eliminate ligand-induced dimerization and increase inhibitor potency 10x.

Keywords(s): SARS-CoV-2, MERS-CoV, CCoV-HuPn, 3CLpro, Nirmatrelvir, ligand-induced dimerization, Structure Activity Relationship

Poster number: 14
Assignment: Session 2 (even number posters) 1-2 pm
Name: Teagan Campbell
Title: Proteomic-based Mechanistic Studies of Anti-Amoebic Cyclic Peptide Agents
Authors: Teagan L. Campbell, Chenyang Lu, Gabriela Coy, Zhuangyan Xu, Miranda Weigand, Gregory T. Knipp, Elizabeth I. Parkinson, Christopher A. Rice, Bryon S. Drown
Abstract: Balamuthia amoebic encephalitis (BAE) is a rare, but often lethal central nervous infection caused by Balamuthia mandrillaris, a pathogenic free-living amoeba (pFLA), with a mortality rate greater than 90%. Although a variety of drugs have been administered by clinicians, in vitro testing has shown that 62% of these agents fail to inhibit B. mandrillaris. This demonstrates the critical need for the discovery and development of novel therapeutics. Cyclic peptides are a developing class of therapeutics that have demonstrated activity across numerous disease states; this is attributed to possessing both characteristics of small molecules and of biologics. Natural product inspired cyclic peptides with improved anti-amoebic activity and minimal cytotoxicity to mammalian cells were identified via phenotypic screening against several pFLA. Despite the promise of these initial selective hit compounds, translation is limited without an identified target and certainty regarding brain penetrance. A triculture transwell assay was used to model blood brain barrier permeability

in which cyclic peptide flux was quantified by LC-MS multiple reaction monitoring. Additionally, time-dependent global and phosphoproteomic studies identified differentially regulated proteins and a strong downregulation of phosphorylated peptides, indicative of specific protein kinase inhibition. Future work includes leveraging protein-level enrichment utilizing photoreactive conjugation to identify drug-protein interactions.

Poster number: 15
Assignment: Session 1 (odd number posters) 11-12 pm
Name: Stephanie Barrios
Title: Investigating the Guanine Nucleotide Exchange Factor Activity of Phospholipase Ce
Authors: Stephanie L. Barrios, Dr. Kadidia Samassekou, Elizabeth E. Garland-Kuntz, Dr. Angeline M. Lyon.
Abstract: Heart failure is the leading cause of death globally, with existing treatments primarily managing symptoms rather than stopping disease progression. Phospholipase Ce (PLCe) is a key regulator of cardiac function and is activated by small GTPases that localize it to either the plasma or perinuclear membranes. At the plasma membrane, RhoA-driven PLCe activity protects heart cells from ischemia-reperfusion injury. At the perinuclear membrane, Rap1A recruits PLCe to enhance cardiac contractility. However, sustained activation through this latter pathway promotes pathological signaling that leads to cardiac hypertrophy and heart failure. This signaling is thought to be due to the CDC25 domain of PLCe, which is a guanine nucleotide exchange factor (GEF) to activate Rap1A. Activated Rap1A binds to PLCe, increasing its lipase activity in a feed-forward loop that drives pro-hypertrophic gene expression. To understand and potentially disrupt this pathological cycle, this study seeks to define the structural and mechanistic basis of PLCe GEF activity, focusing on its specificity for Rap1A and the role of membrane in its activity, using biochemical assays and X-ray crystallography. These insights may guide therapeutic strategies aimed at blocking Rap1A-driven pathways that contribute to heart failure.

Poster number: 16
Assignment: Session 2 (even number posters) 1-2 pm
Name: Ishaan Singh
Title: Enhancing Real Time Precision Opto-Control of Intracellular Activities
Authors: Ishaan K. Singh, Bin Dong, C. Zhang
Abstract: Real-time Precision Opto-Control (RPOC) is a powerful technique for subcellular optical manipulation, enabling targeted application of secondary lasers to selected pixels of a fluorescence image with diffraction-limited spatial precision. Despite its potential in optogenetics and photodynamic therapy, broader adoption of RPOC has been limited by the sophisticated software it requires. We present an open-source, Python-based GUI that integrates RPOC into a full-featured confocal imaging platform, designed to lower the barrier to implementation in biological labs. The software supports multi-channel imaging, Z-stack and hyperspectral scanning, direct control of galvo mirror scanning parameters, and more, all seamlessly paired with the RPOC-specific tools for image-based laser modulation. Modulation masks can be generated automatically via a user-defined preprocessing pipeline or drawn manually, and multiple secondary lasers can be modulated independently and simultaneously during a single imaging cycle. We demonstrate the software's utility by investigating the apoptotic bystander effect in cells with photosensitized mitochondria, leveraging the software's ability for precise irradiation while observing real-time outcomes in untreated neighbors. Ongoing development includes support for automated mosaic imaging

and integrated image processing. As these capabilities expand, the software will continue to bridge the gap between advanced opto-control and general microscopy technology.

Poster number: 17
Assignment: Session 1 (odd number posters) 11-12 pm
Name: Sohan Shah
Title: Towards Efficient Pharmacological Profiling of Clinical GABA-A Receptor Variants by Deep Mutational Scanning
Authors: Sohan Shah and Jonathan Schlebach
Abstract: γ-aminobutyric acid type A receptors (GABA-AR) are ligand-gated ion channels found primarily in the central nervous system. Loss of function mutations can lead to neurological diseases, like epilepsy. Mutations in any of the subunits can cause misfolding and eventual dysfunction through the loss of proteostasis. Maintaining proteostasis is key for cellular function and to avoid protein-based diseases. Proteostasis becomes unmaintained when many proteins are degraded due to the occurrence of misfolding in mutated proteins which can eventually lead to disease phenotypes. Small molecule pharmacochaperones bind and stabilize their targets in order to restore proper protein expression. These compounds take advantage of the connection between folding and ligand binding to reduce thermodynamics hinderances within the protein. Different variants of a protein interact differently with pharmacochaperones where only some see rescue of expression. So, a compound that is effective for one person could be useless for another. Our lab uses deep mutational scanning to study proteins and their variants with respect to this problem. Utilizing a genetically modified cell line that allows for the stable recombination of two plasmids, we have created a scanning platform that allows for high-throughput surface expression measurements for the GABA-AR. To validate this system, we measured expression levels of known clinical variants, and also the effects of Hispidulin and TP003: known GABA-AR interactors that the Ting-Wei Mu Lab has found to act as pharmacochaperones. This system allows for high-throughput studies of GABA-AR and can also be adapted for other oligomeric membrane proteins to evaluate expression levels and other characteristics.

Poster number: 18
Assignment: Session 2 (even number posters) 1-2 pm
Name: Misa Meadows
Title: Better Safe Than Sorry: Preventing the next pandemic by studying newly emerging coronaviruses
Authors: Misa Meadows, Uttara Jayashankar, Monika Yadav, Arun K. Ghosh, Andrew D. Mesecar
Abstract: The effects of the COVID-19 global pandemic caused by the betacoronavirus, SARS-CoV-2, has emphasized the importance of studying newly emerging coronaviruses that have the potential to cause future pandemics. The recent emergence of CCoV-HuPn-2018 (CCoV), a human-infecting canine coronavirus, was detected in patients with pneumonia in Malaysia, identifying CCoV as a zoonotic virus. Upon sequencing, the novel CCoV was classified as an alphacoronavirus, similar to other animal-infecting coronaviruses such as the feline infectious peritonitis virus (FIPV), which infects cats.

Coronaviruses are positive sense, single-stranded RNA viruses that translate their genome into two large polypeptides, pp1a and pp1ab. The 3-Chymotrypsin-like protease (3CLpro) is responsible for cleaving these polypeptides at eleven sites to form smaller, non-structural

proteins that are essential for viral replication. The importance of the 3CLpro and its conservation amongst various coronaviral genera make it an attractive drug target.

The objective of our study is to repurpose small molecule inhibitors designed against SARS-CoV-2 in efforts to establish broad-spectrum inhibition against alphacoronaviruses CCoV and FIPV. In this study, we use a fluorescent-based peptidomimetic substrate to characterize and compare the 3CLpro enzymatic properties. We further performed Structure Activity Relationship (SAR) studies to identify characteristics of the inhibitors contributing to broad-spectrum efficacy. To understand how the identified inhibitors affect the stability of the protein, the melting temperatures of the 3CLpros bound to these inhibitors were quantified using Differential Scanning Fluorimetry (DSF). Together, this study provides valuable information for the development of broad-spectrum inhibitors against multiple coronaviral genera.

Poster number: 19
Assignment: Session 1 (odd number posters) 11-12 pm
Name: Troy Sievertsen
Title: SHIP1 and its Potential as a Therapeutic Target in Late-Onset Alzheimer's Disease
Authors: Troy Sievertsen, Kratika Singhal, and Andrew Mesecar
Abstract: Alzheimer's disease (AD) is a growing global health crisis in urgent need of new therapies. While other high-mortality diseases have seen progress, AD-related deaths have more than doubled. AD is classified into early-onset (under 65 years old, ~5% of cases), associated with high-penetrance mutations, and late-onset (over 65 years old, ~95% of cases), linked to multiple low-penetrance polymorphisms. Accumulation of amyloid-beta (Aβ) plaques is a hallmark of AD and a long-standing therapeutic target. One strategy aims to activate microglia to phagocytose Aβ and other debris. As part of the innate immune system, microglia express TREM2, which binds Aβ oligomers and triggers phosphorylation of intracellular activation motifs to ultimately clear out amyloid beta plaques. These motifs typically recruit kinases that activate signaling pathways utilizing PI(3,4,5)P3. SHIP1, a negative regulator of the TREM2 pathway, suppresses microglial activation by binding these motifs and converting PI(3,4,5)P3 into PI(3,4)P2. Due to its inhibitory role and elevated expression in LOAD patients, SHIP1 is a compelling drug target; inhibiting it may enhance microglial activation and Aβ clearance. We use biophysical methods to uncover new structural and kinetic features of SHIP1 that could inform small-molecule inhibitor design.

Poster number: 20
Assignment: Session 2 (even number posters) 1-2 pm
Name: Nidhi Walia
Title: Borad spectrum screening of nirmatrelvir derivatives against diverse coronavirus 3CL proteases
Authors: Nidhi Walia, Uttara Jayashankar, Sydney Beechboard, Monika Yadav, Arun K. Ghosh, Andrew D. Mesecar
Abstract: Coronaviruses, including SARS-CoV, MERS-CoV, and SARS-CoV-2, continue to pose major global health threats due to their high transmissibility and zoonotic potential. The main protease (3CLpro) from the virus is essential for replication and is conserved the across coronaviral genera, making it a prime target for antiviral development. Although nirmatrelvir, the active component of Paxlovid, is FDA-approved and effective against SARS-CoV-2, it exhibits limited activity against certain other coronaviruses, underscoring the need

for more diversely effective inhibitors. In this study, we performed broad spectrum screening of small molecules targeting 3CLpro enzymes from a range of alpha-, beta-, and gamma-coronaviruses. To overcome the specificity limitations of nirmatrelvir, we rationally redesigned its scaffold by modifying the P1 and P4 subsites, yielding a new series of hit compounds with improved pan-coronaviral inhibition. Lead candidates showed potent enzymatic inhibition across diverse 3CLpro targets, as confirmed by Ki measurements. Thermal stability assays showed all tested 3CLpro enzymes as highly stable under experimental conditions, supporting their suitability for biochemical and structural studies. Ongoing structural studies will help us elucidate the binding modes of these compounds and inform downstream structure-guided drug design. These findings provide a strong foundation for future therapeutic interventions and pandemic preparedness

Poster number: 21
Assignment: Session 1 (odd number posters) 11-12 pm
Name: Katelyn Tepper
Title: The Structural Basis of Arrestin Bias in Neurotensin Receptor 1 Signaling
Authors: Katelyn Tepper, Yasmin Aydin, Kyle Miller, Shuaitong Zhao, Qiuyan Chen
Abstract: Substance use disorders affect many people in their lifetimes, and make up a group of diseases that lack sufficient treatment options. Direct targeting of the dopamine receptor via an agonist as a tool to treat withdrawal symptoms, however, runs the risk of itself having potential for abuse. The neurotensin receptor 1 (NTSR1) presents an alternative target, due to its less direct role in regulating dopamine signaling. The recent development of the biased ligand SBI-553 presents an opportunity to understand the mechanism behind this bias. In NTSR1, a G protein-coupled receptor, this means bias toward the arrestin pathway, rather than canonical G protein signaling. This research aims to determine specific structural interaction changes between SBI-553-bound NTSR1 and the effector arrestin when compared to the complex with the endogenous NTSR1 ligand neurotensin (NTS). So far, our research has shown that the phosphorylated NTSR1 (pNTSR1) interacts differently with arrestin 3 than arrestin 2 when bound to NTS in a fluorescent bimane assay. We have also made progress toward a cryo-EM structure that will help paint a picture of the differences between the endogenous and biased complexes. These structural insights can be used to aid in the development of new NTSR1-targeted therapeutics.

Poster number: 22
Assignment: Session 2 (even number posters) 1-2 pm
Name: Anika Jain
Title: Emap2Lig: Predicting and Modeling ligands in cryo-EM Maps Using Deep Learning
Authors: Anika Jain*1,Shu Li*2,Daisuke Kihara1,2 (*co-first authors)
Abstract: Cryo-electron microscopy (cryo-EM) has revolutionized structural biology by enabling near-atomic visualization of macromolecular complexes, yet automated identification and modeling of small-molecule ligands within density maps remains a significant challenge. We present Emap2Lig, an end-to-end computational framework designed to detect ligand and macromolecular components such as protein backbone , sidechains, nucleotide sugars, phosphates and bases in a given cryo-EM density map in resolutions upto 6 Å. In addition to the detection of the full density of the ligand, the model also detects fragment density of 6 main fragment groups - non-hydrogen tetra and penta-atomic fragments, fused heterocyclic systems, hexacyclic rings, pentacyclic rings and porphyrin-like fragments in the ligand density. We further demonstrated two methods to use

the predicted density information to model the three-dimensional structure of the ligand.The first employs a diffusion-based generative approach, while the second utilizes rigid body docking combined with fragment-based selection criteria. The results show promising performance, with a significant proportion of correctly modeled ligands appearing in the top rankings. This framework advances our ability to interpret ligand-bound structures from cryo-EM data, potentially accelerating structure-based drug discovery efforts.

Poster number: 23
Assignment: Session 1 (odd number posters) 11-12 pm
Name: Sudip Bhowmick
Title: Investigation of Water Splitting Mechanisms in Photosystem II Using Near-IR Resonance Raman Spectroscopy
Authors: Sudip Bhowmick, Roman Ezhov, Hasan Tuncer and Yulia Pushkar
Abstract: Photosynthesis, a process present in algae, green plants, and cyanobacteria, converts sunlight into energy that sustains all living beings on Earth. Photosystem II (PSII) catalyzes the light-driven water oxidation in the process of light to energy conversion. [1]. This reaction is driven by the Mn4Ca complex, which transitions through four stable states (S0 to S4) as described in Kok's cycle, and is embedded in the thylakoid membrane [2]. The exact mechanism of water splitting by the Mn4Ca cluster and the intermediate structural changes of the catalyst during this process are still not fully understood [3]. Previously, some studies using FTIR spectroscopy have attempted to explain this mechanism [4], but Near-IR resonance Raman spectroscopy shows potential for additional approach in understanding of this process. In this study, PS II-enriched thylakoid membranes were prepared from spinach as well as from cyanobacteria [5] and Near-IR resonance Raman spectra (at 830 nm excitation) were recorded from these freshly prepared PS II samples at room temperature and on cryostage. The addition of hydroxylamine solution which reduces Mn4Ca cluster to Mn2+ ions and results in its destruction resulted in changes of the Raman peaks of PS II sample indicating contribution of Mn4Ca cluster vibrations. Two laser pulses were used to transition PS II from the S1 to S3 state, and the corresponding resonance Raman spectra were recorded at nearly 77K temperature. Thereafter, a 50 mM PPBQ solution in DMSO was added as an artificial electron acceptor before excitation using laser pulses to enhance the conversion of the S3 state. The changes in the Raman peaks of Mn in the MnIV oxidation state were analyzed using resonance Raman signal. Additionally, we studied some manganese model compounds, such as [Mn2O2(tPy)2(H2O)2]3+, to identify Mn's Raman signal using the same Near-IR resonance Raman spectroscopy and laser.

Poster number: 24
Assignment: Session 2 (even number posters) 1-2 pm
Name: Danielle McConnell
Title: High-Throughput Screening for Small Molecule Modulators of Phospholipase C β3
Authors: Danielle McConnell and Angeline Lyon
Abstract: Phospholipase C (PLC) enzymes are well-studied cell signaling relays involved in regulating a variety of physiological functions including smooth muscle contraction, chemotaxis, opioid sensitivity, and cell proliferation and survival. Dysregulation of PLCβ3 has been linked with a variety of cardiovascular conditions including hypertrophy and heart failure. Despite its clinical relevance, there are no known selective and direct inhibitors of PLCβ3. Current small molecule inhibitors of PLCβ3 suffer from poor bioavailability, selectivity issues, and detrimental off-target effects. The present study aims to carry out a

high-throughput screen of the commercially available LOPAC1280 library, a structurally diverse collection of compounds with known pharmacological activity. The screen will be conducted using a commercially available in-cellulo IP1 assay that has been well-established for evaluating phospholipase C activity, and stable pools generated from genetically engineered human embryonic kidney (HEK-293) cell lines. Through this screen we aim to identify novel small molecule modulators, enhancing our arsenal of tools for studying PLCβ3 function and potentially allowing for the development of more targeted and effective therapies for cardiovascular disease.

Poster number: 25
Assignment: Session 1 (odd number posters) 11-12 pm
Name: Shaswata Roy
Title: Transcriptional bursting kinetics provide insights into the mechanisms governing Interferon-mediated gene expression
Authors: Shaswata Roy, Manalee Samaddar, Clayton Seitz, Jing Liu
Abstract: Transcriptional memory, where cells exhibit an enhanced transcriptional response upon re-exposure to stimuli, is a key mechanism underlying trained immunity. However, the role of transcriptional bursting in establishing transcriptional memory remains poorly understood. In this study, we investigate how transcriptional bursting kinetics contribute to long-term memory formation in immune responses. We focus on GBP5, a regulatory protein involved in immunity and inflammation, and track its nascent and mature mRNA levels at single-cell resolution over time using single-molecule FISH imaging in HeLa cells subjected to Interferon gamma stimulation. To interpret the overall dynamics, we modeled the upstream signaling pathway driving GBP5 expression and combined it with stochastic two-state models to capture bursty gene activity at shorter time scales. The kinetic parameters were inferred using approximate Bayesian computation and sequential Monte Carlo sampling. To broaden the scope, we also performed Xenium spatial transcriptomics experiments to study the immune response across a range of genes. Our analysis revealed that while transcriptional memory leads to enhanced secondary responses, the transcriptional burst size remains largely unchanged under interferon gamma treatment. Together, these results provide new insights into how transcriptional bursting dynamics contribute to the maintenance of transcriptional memory.

Poster number: 26
Assignment: Session 2 (even number posters) 1-2 pm
Name: Rongxue Zhang
Title: Structural Insights into PDGFR-β Vacancy G-Quadruplex and Fill-in Mechanism
Authors: Rongxue Zhang, Kaibo Wang, Jonathan Dickerhoff, Danzhou Yang
Abstract: PDGFR-β overexpression drives various human diseases including cancer and atherosclerosis. The proximal PDGFR-β promoter forms G-quadruplex that functions as a transcription silencer and is an attractive drug target. The PDGFR-β promoter contains seven G-runs and the major G-quadruplex is formed in the 5'-mid region, which can be stabilized by G4-ligands to downregulate PDGFR-β gene. We determine the folding structure of the major 5'-mid G-quadruplex using NMR in combination with DMS footprinting and mutational analysis. It adopts a novel "broken-strand" structure with a 2+1 discontinuity in K+ solution. The broken-strand structure can be considered as an intramolecular fill-in vacancy G-quadruplex (vG4) with an incomplete G-tetrad. Using NMR in combination with biophysical and biochemical methods, we show that the 5'-

mid broken-strand G-quadruplex forms two equilibrating vG4s. Intriguingly, the PDGFR-β vG4 can be readily filled-in and stabilized by external guanine-derivatives and we determined the structure of the dGMP-fill-in-vG4 in K+ solution. Furthermore, we discovered that the small-molecule medicinal compound berberine binds and stabilizes the dGMP-fill-in-vG4, and we determined the ligand-bound ternary complex structure by NMR. Collectively, our findings provide a structural basis for the potential PDGFR-β transcriptional regulation by guanine metabolites and rational design of drugs targeting PDGFR-β G-quadruplex.

Poster number: 27
Assignment: Session 1 (odd number posters) 11-12 pm
Name: Canyen Setser
Title: Structural and Functional Analysis of Ras Isoforms in Phospholipase Cε Signaling
Authors: Canyen Setser
Abstract: The Ras family of small GTPases plays a critical role in cellular signaling, proliferation, and the regulation of phospholipase Cε (PLCε). PLCε is one of 6 different isozymes in the PLC family. This family of lipases hydrolyze the phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2). This creates two secondary messengers; diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3), which activate protein kinase C (PKC) and calcium channels, respectively. Ras regulates PLCε by binding to the RA2 domain, which is one of two C-terminal Ras association (RA) domains. There are four isoforms of Ras: H-Ras, N-Ras, K-Ras4A, and K-Ras4B. These all share a common G-domain but have different hypervariable regions (HVRs), which contains the membrane targeting region. We hypothesize that the differences in the HVR promote localization of PLCε to substrate-enriched regions of the cell membrane. In this study, we will generate different Ras chimeras using Takara Infusion Cloning and SnapGene. We will test these chimeras in cell-based activity assays to uncover possible mechanisms of interaction and localization of PLCε to the plasma membrane. Our study of Ras isoform-specific interactions with PLCε could provide insights into how Ras signaling influences tumorigenesis in pancreatic, lung, and colorectal cancers and how PLCε leads to cardiac hypertrophy in response to chronic cardiac stress.

Poster number: 28
Assignment: Session 2 (even number posters) 1-2 pm
Name: Dan Xie
Title: Cryo-EM Structure of sisTnpB: Insights into the RNA-guided DNA cleavage mechanism
Authors: Dan Xie[1], Renjian Xiao[1], and Leifu Chang[1]
[1]Department of Biological Sciences, Purdue University, West Lafayette, IN 47907
Abstract: Transposon-associated TnpB proteins are thought to be the ancestral branch of the class 2 type V CRISPR effector Cas121. In bacteria, TnpB proteins are encoded with IS200/IS605 and IS607 family transposons. These transposons have conserved left end (LE), right end (RE) sequences and non-coding RNAs, referred to as ωRNA (Fig.1a)1. TnpB of Deinococcus radiodurans ISDra2 has been identified as an RNA-guided DNA endonuclease1-3 (Fig.1b) and shown a great potential for robust genome editing4. Recent studies demonstrated a new archaea TnpB from Sulfolobus islandicus (sisTnpB) is a thermo-stable endonuclease5and flexible with a broad range of TAM variants6. However,

the molecular mechanism of sisTnpB remains unknown. Here we present the cryogenic-electron microscopy (Cryo-EM) structures of the sisTnpB-ωRNA complex in free and DNA-bound forms. The structures reveal the basic architecture of sisTnpB nuclease and the molecular mechanism for DNA target recognition and cleavage that is supported by biochemical experiments.