VENKATA NARAYANA ARE, PHD **9:15 - 9:35 AM**
POST-DOC - PURDUE UNIVERSITY

CRYO-EM STRUCTURES AND BIOCHEMICAL ASSAYS REVEAL DENGUE VIRUSES UTILIZE HEPARIN AND GLYCAN BINDING RECEPTOR DC-SIGN FOR ENDOCYTOSIS

Dengue virus (DENV) is a member of the genus flavivirus and family Flaviviridae, is transmitted by mosquitoes, that includes four serotypes (dengue 1, 2, 3 and 4). Nearly 400 million people are infected by DENV each year. The rate of infection severity ranges from asymptomatic to mild febrile illness to life-threatening dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). DENV use host cell glycosaminoglycans for attachment, and dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) is a putative receptor. Here we present the first cryo-EM structure of the DENV2-heparin complex at 3.8 Å resolution, the DENV2 DC-SIGN Carbohydrate Recognition Domain (CRD) complex at 3.3 Å resolution and the DENV2 complex with both heparin and DC-SIGN at 3.2 Å resolution. Heparin and DC-SIGN have adjacent binding sites. Heparin hexasaccharide binds to the envelope protein domain II surface via electrostatic interactions with the positively charged residues. N-acetyl glucosamine attached to N67 forms polar interactions with heparin for its stabilization in the binding site. DC-SIGN CRD strictly binds to the envelope protein N67 glycan. Neutralization assays reveal that DENV1, DENV2 and DENV4 serotypes utilize heparin as an attachment factor and DC-SIGN serves as a receptor for initiating clathrin-mediated endocytosis. The absence of heparin on the cell surface and lack of the N67 glycan on the virus results in complete loss of virus infectivity. Our study provides insights into how heparin and DC-SIGN bind DENV and identifies new targets for developing flaviviral entry inhibitors.

RYAN H. GRAY **9:45 - 10:00 AM**
GRADUATE STUDENT - PURDUE UNIVERSITY

USING CRYO-ELECTRON TOMOGRAPHY TO STUDY IN SITU BINDING

The in vitro study of macromolecular binding has been the source of much of our quantitative biochemical knowledge. Despite the many strengths of in vitro binding experiments, they are still limited in their biological relevance by their reliance on steady-state approximations and homogenous environments with infinite volumes relative to the macromolecules. Cryo-electron tomography can provide in situ data without these approximations but is often limited to qualitative assessments or morphological data. By using cryo-electron tomography to identify the spatial distribution and ratio of polymerized to monomeric RuBisCO inside functional α-carboxysomes, it is possible to determine a quantitative polymerization constant in a more biologically relevant context. Here we present guidelines for making biophysical measurements using cryo-electron tomography, and a case study of using these approaches to quantify redox state effects on RuBisCO polymerization.

MERCY ORUKPE MOSES **10:05 - 10:20 AM**
GRADUATE STUDENT - PURDUE UNIVERSITY

INSIGHT INTO DENGUE VIRUS NONSTRUCTURAL PROTEIN 1 (NS1) INTERACTION WITH APOLIPOPROTEIN A1

Dengue virus is the most widespread flavivirus, with about 400 million people infected each year. The dengue virus nonstructural protein 1 (NS1) is known to be involved in genome replication, immune evasion, and inflammatory responses. Recent studies have shown a novel interaction between NS1 and high-density lipoproteins (HDL). In patient serum infected with dengue virus serotype 2 (DENV2), NS1 was seen complexed with Apolipoprotein A1, Apo-A1 (which makes up 70% of HDL) resulting in the depletion of lipid rafts and thereby inhibiting DENV2 attachment to RAW 264.7 cells. The DENV2 NS1 protein was shown to utilize nonpolar interactions to bind directly with the Apo-A1 protein moiety of HDL. The molecular determinants of NS1 binding Apo-A1, the specific site(s) of interaction and how it affects the virus lifecycle remains unknown. In this study, we hypothesize that NS1 interacts with Apo-A1 at specific sites on the DENV2 NS1 hydrophobic regions and this alters viral infectivity. Structural insights into NS1 and Apo-A1 interaction were ascertained using Cryo-electron microscopy. Individual deletions were done in the three hydrophobic regions of NS1, pull down assay, immunofluorescence assay (IFA) and isothermal calorimetry (ITC) were carried out to check for its interaction with Apo-A1. The individual deletions did not affect NS1 interaction with Apo-A1 but altered its affinity in interacting with Apo-A1. To investigate how the presence of Apo-A1 affects dengue virus infection in human cell lines, plaque assays were carried out and pre-transfection/pre-incubation with NS1 and Apo-A1 reduced the infectivity of Dengue 2 virus in Vero cells. However, the overall effect of this interaction in the viral lifecycle is understudied. Our findings from these studies will provide insight into how NS1 interacts with apolipoproteins (Apo-A1) and the resultant effect in the dengue virus infection thereby paving the way for a potential novel drug target.

BARSHA BHOWAL **10:25 - 10:40 AM**
GRADUATE STUDENT - PURDUE UNIVERSITY

EFFECT OF VP40 MUTATIONS ON MEMBRANE ASSOCIATION AND BUDDING OF MARBURG VIRUS

The Marburg virus (MARV) is a zoonotic virus first identified in a highly fatal hemorrhagic fever outbreak in humans and non-human primates in Marburg, Germany, in 1967. Since then, there have been several outbreaks worldwide, the most recent ones being in Equatorial Guinea and Tanzania in early 2023. Human-to-human transmission can occur through direct contact with bodily fluids. There are no specific treatments or approved vaccines for Marburg virus disease (MVD), and supportive hospital care is the only option. However, some repurposed small molecule drugs such as remdesivir and some vaccines are currently under investigation. MARV is a filamentous negative-stranded virus with a genome that encodes seven genes. The matrix protein VP40 is involved in the budding of the virus and immunosuppression. The VP40 N-terminal domain forms the dimer interface, while the C-terminal domain is involved in binding to anionic host cell plasma membrane lipids. VP40 oligomerizes at the plasma membrane inner leaflet, which induces membrane curvature and viral budding. When expressed in the absence of other MARV proteins in mammalian cells, VP40 is sufficient to assemble and bud as non-infectious virus-like particles. Serially passaging of MARV in immunosuppressed mice and Vero E6 cells results in several adaptive mutations6. VP40 actively mutated while maintaining lethality. Our research involves the examination of how some of these mutations (G79S, L96P, E238A, E260A, and E268A) affect membrane localization and virus-like particle (VLP) production from HEK293 cells. We have seen that L96P mutation reduces membrane localization of VP40 significantly and may impact VLP budding. For our next steps, we will purify these proteins and observe if the mutations affect dimerization. This information will elucidate the sequence-structure relation of VP40 and further illuminate VP40-host interactions.

BIN DONG, PHD **1:00 - 1:20 PM**
POST-DOC - PURDUE UNIVERSITY

UNLEASHING PRECISION AND FREEDOM IN OPTICAL CONTROL: SOFTWARE-ASSISTED REAL-TIME PRECISION OPTO-CONTROL OF INTRACELLULAR MOLECULAR ACTIVITIES AND CELL FUNCTIONS

The traditional method in biological science to regulate cell functions often employs chemical interventions, which commonly lack precision in space and time. While optical manipulation offers superior spatial precision, existing technologies are constrained by limitations in flexibility, accuracy, and response time. Here, we present precise optical manipulation of molecular activities and cell behaviors via innovations in human-machine interaction (HMI). A software-assisted real-time precision opto-control (S-RPOC) was developed by integrating adaptive target selection and flexible decision-making. The advanced human-machine interface facilitates automatic target selection driven by optical signals while permitting user-defined delineation. It allows the creation of various optical manipulation conditions in the same field of view and simultaneous monitoring of short-term and long-term cell responses. Specifically, S-RPOC showcases versatile capabilities including adaptive photobleaching, comprehensive quantification of protein dynamics, selective organelle perturbation, control of cell division, and manipulation of individual cell behaviors within a population. By bringing advanced HMI to optical manipulation, S-RPOC holds the promise to advance our knowledge in site-specific biomolecular activities and bring about new approaches to control behaviors of biological samples.

YUEYI CHEN **1:30 - 1:45 PM**
GRADUATE STUDENT - PURDUE UNIVERSITY

STRUCTURAL INSIGHTS INTO A POTENT AND HIGHLY SELECTIVE REVERSIBLE COVALENT GRK5 INHIBITOR FOR TREATMENT OF CANCER AND HEART FAILURE

G protein-coupled receptor kinases (GRKs) regulate cell signaling by triggering receptor desensitization via phosphorylation on G protein-coupled receptors (GPCRs). The seven human GRKs (GRK1−GRK7) are classified into three subfamilies: GRK1 (GRK1 and GRK7), GRK2 (GRK2 and GRK3), and GRK4 (GRK4, GRK5, and GRK6). GRK2 and GRK5 are the most abundant in cardiovascular tissue, where they are potential targets for treatment of cardiovascular disease. GRK5 also undergoes Ca2+/calmodulin-dependent nuclear localization, where it phosphorylates histone deacetylase 5 (HDAC5), inducing an increase in transcription of cardiac hypertrophy-related genes. In GRK5-knockdown mice, cardiomyocytes are protected from hypertrophy; however, the specific roles of GRK5 in heart failure and hypertrophic cardiomyopathy are still unclear. Furthermore, GRK5 is required for cancer progression in various cancer types. Depletion of GRK5 has been shown to suppress prostate cancer, breast cancer, and non-small-cell lung cancer. Therefore, targeting GRK5 can also be a chemotherapeutic strategy. Here we evaluated a series of inhibitors for GRK5/6 derived from the indolinone scaffold, utilizing Cys474 residue unique in GRK5/6 to enhance selectivity by covalent capture. With collaborator, we explored novel inhibitors for improving potency and selectivity without potential toxic functional groups. So far, we have identified several highly potent and selective GRK5 inhibitors. We have developed a unique soaking method to solve GRK5 structure in complex with these novel inhibitors through X-ray crystallography at a sub-3 angstrom resolution. The structure of compound 4c elucidated the binding of this new class of GRK5 inhibitors, where the core interacts with the adenine site, fluorophenyl group stabilizes the P loop and density suggests covalent bond forming between the reversible covalent warhead and Cys474. SAR analysis of 4c and its derivatives explained the basis for selectivity over GRK2 in accordance with the structure. Our ongoing studies focus on further investigation of the reversible covalent inhibition kinetics and optimizations based on SAR. Overall, the discovery of the inhibitors can significantly facilitate understanding and treatment of cardiovascular diseases and cancers.

JACOB H. WAT **1:50 - 2:05 PM**
GRADUATE STUDENT - PURDUE UNIVERSITY

STUDYING PROTEINS IN LIVE CELLS: DEVELOPING AN IN-CELL REVERSE ISOTOPE LABELED INFRARED TECHNIQUE

Understanding a protein's secondary structure in its native environment is key to understanding its function. But while many techniques are available to study the structure of isolated proteins, few options exist to monitor protein structure in live cells. The most common methods for in-cell structural studies are Nuclear Magnetic Resonance (NMR) and Förster resonance energy transfer (FRET). However, NMR is limited to small proteins while FRET provides limited information on protein structure. Fourier Transformed Infrared Spectroscopy (FTIR) is uniquely situated to fill in for these gaps as FTIR can provide information about a protein's secondary structure with no limitation on protein size. When paired with isotope labeling, FTIR can provide information about the conformation of specific amino acids. Here we demonstrate the functionality of our technique through studying a G protein mutant (NuG2b) through using four amino acid labels through both in-cell and purified proteins. Our work shows that we can isolate the same signal both in-cell and in purified protein, allowing us to monitor the conformation of individual protein residues in a live-cell environment.

AUSTIN TEDMAN **2:10 - 2:25 PM**
GRADUATE STUDENT - PURDUE UNIVERSITY

INFLUENCE OF CALNEXIN ON THE PHARMACOLOGICAL PROFILE OF CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR VARIANTS

Cystic Fibrosis (CF) is a disease caused by mutations to the Cystic Fibrosis Transmembrane Conductance Regulator chloride channel (CFTR), with the most prevalent and well-studied being ΔF508. A treatment consisting of a potentiator and two pharmalogical correctors has been developed and is effective for most but not all variants. I will detail how these correctors interact with an endogenous molecular chaperone Calnexin (CANX) in the expression of 235 CF variants using deep mutational scanning. My results reveal that CANX is required for robust CFTR expression across all variants, but especially for those that perturb the later CFTR assembly transitions. CANX also appears to differentially affect the pharmacological response by enhancing the pharmacological rescue of poorly expressed variants while suppressing the response of highly expressed variants. CANX did not uniformly alter the selective preference for some CF Variants of one compound over another including in a novel selectivity filter found in the N-terminal Lasso region. However, it did eliminate the preference for VX-445 for certain variants found in a domain swapped region of Membranes Spanning Domain 2 (MSD2). This work demonstrates the role of CANX as a late-acting chaperone which attenuates the surface expression of CFTR variants.

YING H. SU, PHD **9:00 - 9:20 AM**
PROFESSOR - UNIVERSITY OF ILLINOIS, CHICAGO

MAPPING IMMUNE CELL MEMBRANES USING A SINGLE-MOLECULE LENS

The intricate network of immune cells and diverse biomolecules that summon, rile, calm, and transform these cells create a complex puzzle. Solving this puzzle requires sensitive tools at the molecular and nanoscale level. In this talk, I will present our development of unique single-molecule and superresolution techniques and applications of these techniques to study specialized membrane structures of immune cells. I will introduce a new superresolution technique using single-molecule labeling. We successfully demonstrated this technique using several labeling agents, including full-length antibodies, antibody fragments, a bicyclic heptapeptide, and semiconductor quantum dots. The new imaging capability allows us to super-resolve mechanically fragile membrane structures. We discovered a massive membrane fiber network that dendritic cells (DCs) utilize for antigen uptake. We further super-resolved filamentous actin (F-actin) in these membrane fibers and discovered their prevalence in immature DCs. Treating DCs with lipopolysaccharide, a potent stimulator, reduced actin-associated membrane fibers. In addition, F-actin spatially rearranged into more defined cytoskeletal structures near the cortex of mature DCs, suggesting an interplay between F-actin and the plasma membrane in regulating the antigen presentation function of DCs. I will conclude with our recent superresolution data suggesting the connection between T-cell microvilli and extracellular vesicles for intercellular communications. Together, ultrasensitive imaging techniques provide unique mechanistic insights toward an enhanced understanding of how immune cells communicate through specialized membrane structures, one molecular piece at a time.

ALEXANDER BAENA **9:30 - 9:45 AM**
GRADUATE STUDENT - PURDUE UNIVERSITY

NOVEL SPOROCARP PRODUCTION SYSTEM USING ENGINEERED BIOPHYSICAL ELEMENTS WITHOUT SOLID SUBSTRATES

Novel biotechnological approaches are needed to enhance renewable food production and waste recycling capabilities in resource-constrained environments. Edible mushrooms possess nutritious profiles and represent emerging opportunities to develop circular bioproduction by transforming waste organic materials into food and high-value products. However, traditional mushroom cultivation methods utilizing solid substrates have technical limitations like 1) uneven nutrient diffusion, 2) constant sterilization needs, 3) numerous intermediate steps that are energy and time-demanding, and 4) waste production from bags and jars. This work introduces an innovative hydroponic fungal cultivation system called Mycoponics™ using bioengineered ceramic materials. This liquid-nutrient-based technique optimizes fungal metabolism and facilitates precise nutrient delivery through biophysically engineered ceramic growth elements, overcoming the limitations of traditional solid substrates. To validate Mycoponics™, we have developed different geometries of the biophysical ceramic growth elements (i.e. mycoponic tubes) and formulated novel living nutrient solutions for experiments using various commercial fungal strains and growth conditions. Sporocarp (i.e. mushroom) production is achieved through different nutrient solutions engineered for the nutritional requirements of each fungal strain. Infrared imaging and microscopy demonstrated consistent, robust mycelial development compared to traditional methods, even when the nutrient solution is not sterilized likewise allowing mycelium access to circulating nutrients without substrate penetration showed longer constant production, avoiding nutrient depletion seen in conventional cakes. Synthesis of high-value products like mycoleather was explored, showing uniform, high-quality material production. By avoiding bulky substrates and exploring new growth surfaces, Mycoponics™ represents a solution for food security, sustainable biotechnology, and bioregenerative life support systems on Earth.

BADEIA SAED **9:50 - 10:05 PM**
GRADUATE STUDENT - UNIVERSITY OF ILLINOIS

IMAGING VESICULAR DYNAMICS AND INTRACELLULAR IL-2 IN ACTIVATED JURKAT T CELLS

Intercellular communication orchestrated by T cells is tightly regulated through cytokine secretion. While effective therapies for cytokine imbalance are available, they lack the potential to modulate and target multiple proinflammatory cytokines. Direct visualization of vesicular transport and intracellular distribution of cytokines provides valuable insights into the temporal and spatial mechanisms involved in regulation. Using Jurkat E6.1 T cells and interleukin-2 (IL-2) as a model system, we studied vesicular dynamics using single particle tracking and the intracellular distribution of IL-2 in fixed T cells by utilizing superresolution microscopy. Following in vitro activation, increased vesicular dynamics were observed using live cell imaging. Direct stochastic optical reconstruction microscopy (dSTORM) revealed an accumulation of IL-2 nanoclusters into more pronounced, elongated clusters. These observations of accelerated vesicular transport and spatial concatenation of IL-2 clusters may provide insights regarding potential mechanisms for modulating cytokine release. An enhanced understanding of the cytokine release mechanism holds great therapeutic promise for addressing cytokine imbalance.

ZHENXIANG ZHAO **10:10 - 10:25 PM**
GRADUATE STUDENT - UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

ACTIVITY-BASED SENSING REVEALS ELEVATED LABILE COPPER ACTIVITY DURING LIVER AGING

Oxidative stress is one of the major culprits of aging and age-related disease states. Although the body features numerous antioxidant defense systems to safeguard against this, conditions ranging from neurodegeneration and cancer to organ failure may result once the buffering capacity of these protective mechanisms have been exceeded. While it is known that redox active metal ions such as copper (Cu) can generate reactive oxygen species (ROS), establishing a possible connection between Cu dysregulation and aging, especially in an in vivo context is exceedingly difficult. Through the development of new activity-based imaging probes for Cu(I) exhibiting ultra-sensitivity, we discovered that labile hepatic Cu activity increases during aging, and this results in the depletion of hepatic stem cells (hSCs) as measured by the ALDH1A1 stemness biomarker. Further, the application of a glutathione (GSH)-responsive probe for noninvasive photoacoustic imaging revealed that these findings are linked to an age-dependent decrease of hepatic GSH activity. To delay this phenotype, we designed two longitudinal studies where aged-mice were treated with ATN-224, a Cu-chelating drug. Our results suggest that treatment restores Cu homeostasis which may have a hSC-sparing effect to delay the onset of liver aging.

PHILLIP S. RUSHTON, PHD **2:00 - 2:20 PM**
POST-DOC - PURDUE UNIVERSITY

FUSING A STAR INHIBITOR OF THE BACTERIAL MEVALONATE PATHWAY FROM A NEBULAE OF FRAGMENTS

Multi-drug resistant bacteria infect millions of people per year leading to billions of dollars in clinical costs and lowered quality of life. With fewer antibiotic drugs in development and fewer reserve antibiotics available to treat infections there is a growing need for drugs that target novel modes of action. With the discovery that the bacterial mevalonate pathway is essential for pathogenic gram-positive bacteria and possesses distinct elements from evolutionarily higher mevalonate pathways it was hypothesized that drugs could be designed to specifically target this pathway with a novel mode of action. This includes the CDC's serious threat level ranked gram-positive strains of Vancomycin Resistant Enterococcus faecalis (VRE), drug-resistant Streptococcus pneumoniae and Methicillin Resistant Staphylococcus aureus (MRSA). Our lab has screened thousands of compounds against HMG-CoA reductase (HMGR), the rate limiting enzyme of the mevalonate pathway, which led to the discovery of a compound possessing low micromolar inhibitory constants against E. faecalis HMGR. Through iterative design phases guided by enzyme kinetics and X-ray crystal structures of HMGR bound with compounds it was possible to synthesize successive generations of better inhibitors. In vivo anti-bacterial experiments revealed many of these compounds can inhibit bacterial growth at low micromolar concentrations against VRA and MRSA strains. 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 homolog crystals to evaluate possible modes of inhibition. Informed by these structures, a more effective antibiotic can be generated with lower inhibitory constants and effective bacterial control. This drug could be used to fight some of the worst bacterial infections humanity suffers.

NICHOLAS L.F. GALLINA **2:30 - 2:45 PM**
GRADUATE STUDENT - PURDUE UNIVERSITY

RECEPTOR TARGETED NEXT-GENERATION PROBIOTICS AMELIORATE GUT INFLAMMATION

Background: Intestinal barrier mis-localization, inflammation and elevated expression of heat shock protein 60 (Hsp60) are features of inflammatory bowel disease (IBD). Probiotics can alleviate IBD, however, they are ineffective due to poor adhesion and adaptation to the diseased bowel. We hypothesize that enhancing probiotic adhesion to intestinal cells may augment the immunomodulatory response, mucosal healing and tight junction restoration. Earlier, we identified Listeria adhesion protein (LAP; 94-kDa) that aids in Listeria attachment to the epithelial cells by interacting with Hsp60. Next-generation bioengineered Lactobacillus probiotics (BLP) expressing LAP from L. innocua (LbcLAPLin) showed strong interaction with epithelial Hsp60, high immunomodulatory response and epithelial barrier integrity. Method: BLP feeding would ameliorate colitis in a dextran sulfate sodium (DSS)-treated mouse model.
Results: DSS (2%,7 days) in water ad-libitum to mice. Mice received BLP in water for 10d and showed a reduction in FITC-labeled 4 kDa dextran (FD4) translocation compared to LbcWT or H2O group. BLP fed DSS-treated mice gained 5% body weight during 10d of BLP feeding when compared to the DSS treated mice that did not receive any probiotics during that period. BLP feeding conferred a 40% reduction in disease activity index when compared to the H2O and 33% to LbcWT mice. BLP treatment restored fecal consistency to Type 3- 4 (Bristol scoring) within 9d of feeding, while the H2O and LbcWT groups failed. Colon examination showed shortening, abdominal adhesions and mucus accumulation in H2O and LbcWT groups while the cecum and colon of BLP mice were improved. Pathologist scoring indicated colon pathology of the BLP-fed group to be 50% lower than the LbcWT while reducing neutrophil counts. Microbiome data shows a partial restoration of diversity and richness, primarily a distinct subpopulation fed with the BLP.

DOROTHY DROZARIO **2:50 - 3:05 PM**
GRADUATE STUDENT - PURDUE UNIVERSITY

STRUCTURAL INSIGHTS INTO CONTACT-DEPENDENT INHIBITION IN BACTERIAL WARFARE

Contact-dependent inhibition (CDI) is a mechanism in Gram-negative bacteria that inhibits neighboring cell growth through direct contact. It involves specialized systems comprising a toxin, an immunity protein, and a receptor. Upon contact, the producing bacterium delivers the toxin into target cells, where it disrupts essential processes, leading to growth inhibition or cell death. The immunity protein protects the producer from self-intoxication, and specificity is ensured through receptor recognition. CDI provides a competitive advantage to the producer and influences microbial community dynamics. We aim to structurally characterize the CdiA protein implicated in the interaction between inhibitory and target bacteria and to elucidate the mechanism of inhibition. Our focus lies on understanding the molecular interactions of two CDI systems: (i) CdiA EC93 with the BAM complex, and (ii) CdiA EC536 with OmpC/F. We have cloned and purified the target proteins of CdiA, namely the BAM complex, OmpC, and OmpF. The receptor-binding domains (RBD) of CdiA-EC93 and CdiA-EC536 were cloned and expressed in BL21(DE3) cells. Despite both constructs yielding proteins in the form of insoluble inclusion bodies, we successfully refolded and further purified them for forming complexes with CdiA. Our size-exclusion chromatography (SEC) assay and native PAGE results demonstrate that CdiA-EC93 interacts with the BAM complex, while CdiA-EC536 interacts with both OmpC and OmpF, forming stable complexes. We are currently performing structural studies of these complexes utilizing X-ray crystallography and cryo-EM, which will serve as a foundation for probing the mechanism these CdiA proteins use during bacterial warfare.

LUKE PISZKIN **3:10 - 3:25 PM**
GRADUATE STUDENT - UNIVERSITY OF NOTRE DAME

NMR RING CURRENT CHEMICAL SHIFT PERTURBATIONS REVEAL FUNCTIONAL DYNAMICS OF OXA 24/40

The internal dynamics of Class-D beta-lactamases have often been implicated in their capacity for antibiotic degradation. However, information on the specific internal motions that are necessary for their catalytic activity is scarce. Recent developments in real-time NMR methods have provided an opportunity to probe the molecular reorientations that occur during the active processes of biomolecules. By comparison with all-atom MD simulations of the carbapenem hydrolyzing Class-D beta-lactamased OXA 24/40, we predicted the ring-current effects due to the dynamics of a critical active site tryptophan (W221) that match well with the real-time experimental changes in active site amide and methyl proton chemical shifts during doripenem turnover. Investigating the functional dynamics of OXA 24/40 is a crucial step for developing a comprehensive plan to combat runaway antibiotic resistance.