KU THE UNIVERSITY OF KANSAS

CHEMICAL BIOLOGY SYMPOSIUM 2025

OCT 10-11

Keynote Lecture – Friday, October 10th

Dr. Bryan C. Dickinson

Professor of Chemistry

Department of Chemistry

University of Chicago

Chicago, IL, 60637

Disorder-driven Molecular Glues

Bryan C. Dickinson

Department of Chemistry, *University of Chicago, Chicago, IL, USA*

Developing molecules that induce proximity between two proteins to modulate their activity is a powerful approach for cell engineering and therapeutics. Bifunctional molecules, which bind two separate targets independently to bring them into proximity, are relatively straightforward to engineer, but suffer from the "hook effect", which makes their biological deployment challenging. Molecular glues can circumvent the hook effect by cooperatively binding the two targets, but their dependence on inherent interactions between targets renders their discovery challenging and limits their broader applicability. In this talk, I will present dynamic bifunctional glues ("disordered glues") that use disorder-to-ordered transitions to drive cooperativity between two separate interfaces as a mechanism to create molecular glues. Discovery of disordered glues uses a novel pipeline that implements our recently developed PANCS-binder technology[1,2] for peptide binder discovery followed by PANCS-glues[3], a new high-throughput screening platform that mies large peptide libraries for molecules that function directly as molecular glues. The dynamic bifunctional glue mechanism, combined with PANCS-glues discovery technology, offers a versatile design strategy for creating cooperative glues for targets of interest, while highlighting how disorder can be harnessed as a powerful tool for molecular design.

[1] Pu *et al.*, *Nature Chemical Biology,* **2017**, *13*, 432.

[2] Styles *et al.*, *Nature Methods, accepted* **2025**. https://www.biorxiv.org/lookup/doi/10.1101/2025.01.06.631531

[3] Wei et al., *In revision* **2025**.

Lightning Talks – Friday, October 10th

Reduced Cholesterol Slows Catalysis by γ-secretase via Stalled Enzyme-substrate Complexes: Implications for the Pathogenesis of Alzheimer's Disease

Tristan A. Sprague[1], Michael S, Wolfe[2]

[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; [2]Department of Medicinal Chemistry, University of Kansas, Lawrence, KS, USA

Mutations in the protease γ-secretase cause familial Alzheimer's disease, a rare, dominantly inherited form of Alzheimer's. However, it is not known how dysfunctional γ-secretase results in the common form of the disease, sporadic Alzheimer's. Previously, we showed that familial Alzheimer's mutations in γ-secretase act by stalling γ-secretase after it forms an enzyme-substrate (ES) complex. γ-Secretase is a membrane protein, and should be sensitive to changes in the membrane itself. We hypothesized that γ-secretase ES complexes can also stall if the membrane's lipid composition is altered. Using purified γ-secretase in artificial liposomes, γ-secretase's proteolytic activity dramatically increases with increasing cholesterol concentration. Furthermore, we show that decreasing membrane cholesterol does not affect APP's affinity for γ-secretase, but does reduce the enzyme's k_{cat} more than 10-fold. We also show putative ES complex stabilization in cells after cholesterol depletion with either statins or β-cyclodextrins, using a fluorescence lifetime imaging approach in cultured cells. These results establish a possible role for stalled γ-secretase ES complexes in sporadic Alzheimer's, a potential mechanistic similarity between the two forms of disease.

Optimized Methods for Large-volume Sample Preparation in Clinical Proteomics

Ashten Gentry[1,2,*], Caitlin McEntee[1,2,*], Samson Aruna[1,2], Michaella Rekowski[3], Thomas Gremminger[3], Rebecca Whelan[1,2]

*[1]Department of Chemistry, University of Kansas, Lawrence, KS, USA; [2]Ralph N. Adams Institute for Bioanalytical Chemistry, University of Kansas, Lawrence, KS, USA; [3]Mass Spectrometry and Proteomics Core Facility, University of Kansas Medical Center, Kansas City, KS, USA, *These authors contributed equally to this work.*

Though blood-based biofluids are commonly used in research, they are difficult to analyze through mass spectrometry assays. The wide dynamic range of protein concentrations can make it difficult to detect signals arising from low-abundance analytes, which include most proteins used as clinical biomarkers. To overcome this problem, many proteomics workflows employ sample enrichment or depletion, which aims to partially eliminate highly abundant proteins while enriching the signal from low-abundance biomarkers. However, many sample enrichment and depletion workflows can cause high dilution of samples. The resulting large volumes require long processing times using currently available platforms, limiting throughput. Reduction of sample volume is possible, but leads to variable sample loss, and can be problematic for low-abundance biomarkers. In this presentation, we report an optimized method for large-volume sample processing prior to bottom-up proteomics, utilizing the commercially available miniprep platform as a filter-based digestion approach. We demonstrate that

desalting is not required for this method. Taken together, the methods reported here enable higher peptide yields and reduced sample processing times while maintaining the integrity of mass spectrometry data collection, as indicated by the number of proteins identified.

PEP-R619W Distinctly Modulates Type-I Interferon Signaling to Enhance DC and CD8 T Cell Function

Anam F. Shaikh, Jenna Barnes, Nancy Schwarting, Tammy Cockerham, and Robin C. Orozco
Department of Molecular Biosciences, Graduate Studies, University of Kansas, Lawrence, KS, USA

Persistent Type I interferon (IFN-I) signaling contributes to chronic viral infections by driving dendritic cell (DC) dysfunction and T cell exhaustion. The autoimmune-associated protein PEP-R619W alters immune responses, but its role in viral infections remains poorly defined. **We hypothesize that PEP-R619W enhances antiviral immunity by modulating IFN-I signaling and boosting DC immunostimulatory protein expression.** Using CRISPR/Cas9-engineered PEP-R619W mice, we show that these mice clear Lymphocytic choriomeningitis virus clone 13 (LCMV-clone13) whereas wild-type (WT) mice do not. Post-infection, PEP-R619W DCs exhibit reduced infection, increased CD86, and decreased PD-L1 expression, both in-vivo and ex-vivo. Transcriptome analysis reveals distinct gene expression in PEP-R619W DCs, with upregulated Jak3 expression. Our finding suggests that PEP-R619W enhances IFN-I signaling as observed by increased pSTAT1 expression and elevated expression of Interferon stimulated genes in DCs. However, PEP-R619W CD8 T cells demonstrate reduced IFN-I signaling, which may contribute to decreased exhaustion. Consistently, PEP-R619W CD8 T cells exhibit more IFNγ and Granzyme B production, specifically in response to the minor epitope of LCMV-cl13 (gp276-284). These findings highlight PEP-R619W as a modulator of IFN-I signaling, preventing immune dysfunction and promoting viral clearance through cell-type-specific effects, offering a potential therapeutic approach for chronic infections.

Gut-microbiome-derived Metabolites Modulate *Vibrio cholerae* Interactions with Host Cells

Saeideh Nasiri and Caetano Antunes

Department of Molecular Biosciences, The University of Kansas, Lawrence, KS, USA;

Microorganisms in the human gut are recognized for their protective roles against pathogens, yet the mechanisms underlying these interspecies interactions remain incompletely understood. Investigating these dynamic interactions is essential to uncover how the microbiome influences pathogen behavior. Our study focuses on *Enterocloster citroniae*, a gut commensal that has been previously shown to produce bioactive compounds that affect *Salmonella enterica* and *Vibrio cholerae* behavior.

Here, we demonstrate that small molecules produced by *E. citroniae* significantly enhance biofilm formation and reduce swimming motility in *V. cholerae*.To assess the impact of bioactive small molecules on host-pathogen interactions, we infected human

colorectal epithelial cells (HT-29) with *V. cholerae* grown in the absence or presence of small molecules produced by *E. citroniae*. Our data indicate that *E. citroniae* produces compounds that inhibit *V. cholerae* adhesion to and invasion of host cells. Initial attempts to isolate the bioactive compound using reverse-phase High-Performance Liquid Chromatography (HPLC) were unfruitful. However, normal-phase HPLC yielded fractions with potent biofilm-inducing activity. Current efforts focus on characterizing these bioactive fractions using mass spectrometry and nuclear magnetic resonance spectroscopy to elucidate chemical structures of bioactive compounds. To this end, we used untargeted metabolomics to characterize the secretome of *E. citroniae* and found several small aromatic metabolites, some of which have well described roles as signaling molecules in other organisms. We are currently testing the effect of specific metabolites for their role in modulating *V. cholerae* biofilm formation.

This study sheds light on the chemical biology of the gut microbiota and its ability to influence *V. cholerae* behavior, providing a foundation for future research into the interplay between pathogens, hosts, and the commensal microbiota.

Mechanisms of Lifespan and Healthspan Benefits by Neuromodulating Drugs on Aging and Age-related Diseases

Shelby A. Innes, Iryna Graham, Mingyi Liu, Jane Oltjen, Joey Kyle, Shijiao Huang

Department of Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS, USA

Aging is the primary risk factor for many age-related diseases including neurodegenerative diseases and cancer. *C. elegans* are a small nematode worm with simple anatomy, short lifespans, and easily modifiable genomes, making them a useful tool in studying aging and age-related diseases. The goal of my research project is to identify neuromodulating compounds that increase the lifespan and improve the healthspan. Compounds can be screened for their ability to increase lifespan of *C. elegans* through analyzing their ability to induce the pro-longevity marker *fmo-2*. We have shown that trifluoperazine and thioridazine, dopamine antagonists, and mianserin, a serotonin antagonist, induce the pro-longevity gene *fmo-2* and increase the lifespan and healthspan of *C. elegans*. All three neuromodulating drugs increase the short-term wildtype *C. elegans* as they age. The three neuromodulating drugs were found to decrease total lipid content. RNAi of genes involved in fatty acid metabolism and transport were utilized to help determine a mechanism by which the drugs induce *fmo-2* and improve healthspan. Gas chromatography and lipidomics experiments have shown differences in specific lipids and fatty acids upon trifluoperazine. Future experiments include lifespan assays with the neuromodulating drugs treatment under RNAi knockdown of genes in fatty acid metabolism and transport, as well as gas chromatography and lipidomics experiments after treatment with mianserin and thioridazine.

Keynote Lecture – Saturday, October 11th

Dr. Christopher G. Kevil

Director/Principal Investigator for Center of Redox Biology and Cardivascular Disease COBRE and Professor of Departments of Pathology, Molecular and Cellular Physiology, and Cellular Biology and Anatomy

Departments of Pathology, Molecular and Cellular Physiology, and Cellular Biology and Anatomy

Louisiana State University Health Shreveport

Shreveport, LA, 71103

Reactive Sulfur Species: the 'missing link' in redox pathophysiology

Christopher G Kevil

Department of Pathology and Translational Pathobiology LSU Health Sciences Center – Shreveport, Shreveport, LA, USA

Reactive sulfur species (RSS) have emerged as a critically important redox biology mediator of numerous cellular and pathophysiological responses from cardiovascular, immunological, neurological, carcinogenesis, and many others. Importance of these mediators is also increasingly appreciated in aging and metabolic dysfunction. Here I present RSS formation from transsulfuration and other enzymes, and their metabolism and molecular function. We have further revealed how cellular hypoxia and tissue ischemia regulates production of RSS persulfide and polysulfide via posttranslational regulation of cystathionine gamma lyase involving differential phosphorylation and prenylation. Together, these responses have significant implications for tissue function and viability and serve as novel molecular therapeutic targets.

Invited Speakers – Saturday, October 11th

A Drug Repurposing Screen Reveals Potential Therapies for the Rare Glycosylation Disease, DPAGT1-CDG

Hans M. Dalton[1], Naomi J. Young[2], Alexys R. Berman[2], Kaylee A. Patterson[2], Sydney J. Peterson[2], Heather D. Evans[2], Clement Y. Chow[2]

[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; [2]Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT, USA

The N-glycosylation pathway adds essential sugar modifications to proteins. Deleterious mutations in glycosylation genes underlie Congenital Disorders of Glycosylation (CDGs): ultra-rare disorders that cause debilitating, multisystemic symptoms. There are few treatment options available for CDGs and little known about the molecular mechanisms involved. Drug repurposing screens, which use libraries of FDA-approved drugs, can find potential therapeutics. Here I will present a completed drug repurposing screen on the rare disease DPAGT1-CDG.

DPAGT1-CDG is a multisystemic disorder with severe developmental and CNS disorders. It is caused by mutations in the gene *DPAGT1*, which encodes for the first essential enzyme in N-linked glycosylation. I created an eye-based model of DPAGT1-CDG in *Drosophila* that causes a small, rough eye phenotype. Screening with a drug repurposing library of 1,520 compounds, I identified 42 candidate drugs that rescued the DPAGT1-CDG model eye size. The strongest drug class was dopamine receptor D2 (D2R) antagonists. Knockdown of D2R mimicked the drug and strongly improved eye size. D2R acts oppositely to the dopamine 1 receptor (D1R), and a heterozygous null of D1R worsened eye size and reduced the improvement of D2R knockdown. Finally, knockdown of dopamine synthesis and recycling pathways also rescued the DPAGT1-CDG model. Thus, the loss of dopamine flux, and its subsequent binding to D2R, improved the impairment of *DPAGT1*. In addition to dopaminergic drugs, I validated acetylcholine-affecting drugs, COX inhibitors (NSAIDs), and an ion transporter (NKCC1)-related drug, as well as a negative hit, an antihistamine. These pathways represent novel biology related to *DPAGT1*, and they may underlie new treatments for DPAGT1-CDG.

NMR Spectroscopic Characterization of the Surface Chemistry and Morphology of mRNA-Lipid Nanoparticle Vaccines.

Justin T. Douglas

Nuclear Magnetic Resonance Core Lab, University of Kansas, Lawrence, KS, USA

The Moderna and Pfizer/BioNTech COVID-19 vaccines demonstrated the power of mRNA-lipid nanoparticles (LNPs) as a rapid, flexible and effective vaccine platform for combating emerging infectious diseases. Preparedness for future pandemics hinges on mRNA-LNP vaccine production in currently underserved low- and middle-income

countries, which will require reducing production costs and improving stability of the liquid dosage form. Achieving this goal will require readily-accessible "developability assessments," a variety of biochemical, biophysical and cell-based assays to measure the key properties that contribute to *in vivo* efficacy and can assess the integrity and quality of the mRNA biomolecule, each of the lipid components, and the combined intact mRNA-LNP. This presentation will describe the results of a recent collaboration between the KU Vaccine Analytics and Formulation Center (VAFC) and the KU Nuclear Magnetic Resonance (NMR) core lab aimed at deploying "developability assessments" for mRNA-LNPs. Scientist at the VAFC employed a number of assays as part of their "routine analytical toolbox" to measure the physicochemical stability profiles of the four mRNA-LNPs as a function of different environmental stresses. NMR spectroscopy was employed to assess the surface chemistry and morphology of the mRNA-LNPs. The key to using NMR in this assay is that in aqueous formulations, molecular components with sufficient mobility (*e.g.,* those present on the solvated surface) will give rise to "detectable" resonance peaks, whereas, the reduced mobility of moieties in the tightly packed internal core render these resonances "invisible" by NMR. The individual components can be characterized via a disruption assay in which the macromolecular structure is perturbed via reconstitution into a non-polar solvent. The combination of the "routine analytical toolbox" with high resolution NMR characterization uncovered key structural attributes (*i.e.,* bleb-like morphology and surface topology) that predict the stability profiles of mRNA-LNP formulations.

Molecular Mechanisms of Neural Targeting of Aging and Age-related Diseases

Shijiao Huang, Shelby Innes, Iryna Graham, Mingyi Liu

Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS, USA

Neurons perceive environmental stress, project to peripheral tissues, and systematically control health and longevity. However, our knowledge of the neuronal connectivity, the metabolic remodeling, and the cross-tissue communication that promote both lifespan and healthspan is still limited. Our previous work identified that neurotransmitters serotonin and dopamine modulate dietary restriction mediated longevity in response to food odor, which can be mimicked by serotonin and dopamine antagonists. We recently further identified multiple neuromodulating compounds that target a wide-range of neurotransmitters consistently extend lifespan and healthspan of both wild-type and neurodegenerative disease models of *Caenorhabditis elegans*. We will use the tractable model organism *C. elegans* to identify the common neuronal mechanisms and metabolic pathways targeted by neural perturbations that convey benefits of both lifespan and healthspan extension. We showed that genes in the pathways of fatty acid and lipid metabolism are required for the health benefits of neuromodulating compounds. We will utilize these compounds as tools to interrogate the common mechanisms of neuronal perturbations in regulating neuronal hemostasis and metabolism remodeling in delaying aging and improving proteotoxicity related neurodegenerative diseases.

Sex Specific Impact of O-GlcNAcase Inhibition on Brain Metabolism

Chad Slawson, Antonio Artigues, and Ibtihal Alghusen

Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS, USA

Multiple different O-GlcNAcase (OGA) inhibitors are in Phase I or II clinical trials as a potential therapeutic target to increase Tau O-GlcNAcylation and reduce phosphorylation in Alzheimer's disease (AD). O-GlcNAc is a single sugar post-translational modification processed by O-GlcNAc Transferase (OGT), which adds the sugar, and OGA that removes the sugar from proteins. Nevertheless, our understanding on how OGA inhibitors impact energetics in AD is limited. Using multiplexed quantitative mass spectrometry, we charted the remodeling of the mouse brain mitochondrial proteome and phospho-proteome during long-term O-GlcNAcase inhibition using Thiamet G (TMG). Interestingly, our analyses reveal that TMG has a gender specific effect with male mice showing abundant protein expression changes. Proteomics identified significant elevations in antioxidant proteins in male with significant reduction in female TMG-treated mice. Mitochondrial electron transport chain (ETC) proteins were significantly decreased in TMG treated male brain's mitochondria compared to saline with almost no change in female. TMG increases complex I activity and decreases complex II activity in males with no change in females. Consistently, mitochondrial hydrogen peroxide (H_2O_2) and superoxide (O_2) were significantly decreased in male, while increased in female. Collectively, our work indicates that OGA inhibition has a profound effect on cellular energetics, ROS production, and metabolic output in a sex specific manner. These data suggest that the usage of OGA inhibitors clinically would cause dramatic mitochondrial proteome reprograming in a sex-specific manner.

HSV-1 ICP0 Dimer Mutants Impair Viral Replication and Protein Activities

Erick McCloskey, Hongping Hao, Rohan Joseph, Chris Oral, and David J. Davido

Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

Infected cell protein 0 (ICP0) is an immediate-early E3 ubiquitin ligase of herpes simplex virus 1 (HSV-1) that performs many functions, in part, through its C-terminal dimer domain (residues 555-767). Deletions in this dimer domain result in reduced viral gene expression, decreased lytic infection, and impaired reactivation from latency. We wanted to determine the structure of this domain, and how the structure modulates known functions of ICP0. X-crystallography analyses of the dimer domain revealed that two monomers of ICP0 form two β-barrel-like motifs held together by an extensive network of hydrogen bonds. Specific amino acids involved in hydrogen bonding were substituted to alanine to prevent dimerization, with the goal of preserving other functional domains in ICP0's C-terminus. Promoter-reporter assays suggest that ICP0 dimer mutants are unable to efficiently stimulate HSV-1 promoters to the same degree as wild-type (WT) ICP0. ICP0 dimer mutants are also unable to fully complement the replication of an ICP0-null mutant. Several ICP0 dimer mutant viruses were generated, and viral yield assays suggest that their replication is significantly reduced in multiple cell types

compared to WT HSV-1. Interestingly, these mutant forms of ICP0 show increased stability during infection compared to WT ICP0 in cell culture. Immunofluorescence assays suggest preservation of the ability for dimer mutant ICP0 to disperse PML similarly to WT ICP0. Lastly, an initial in vivo experiment shows that one ICP0 dimer mutant appears to be impaired for viral replication in the trigeminal ganglia (TG) of mice at 5 days post-infection compared to WT HSV-1 following ocular infection. These results suggest that dimerization alters ICP0's function to enhance aspects of productive infection. Future studies will examine how ICP0 dimerization regulates its auto-ubiquitination activity and ability to bind SUMO modifications and other partners during the HSV-1 lytic cycle.

New Chemical Tools to Study Carbohydrate-Protein Interactions

Matthew C. Russolillo, Wariya Nirachonkul, Patrick A. Ross, Rahul M. Hedau, Mark P. Farrell

Department of Medicinal Chemistry, University of Kansas, Lawrence, KS, USA

The interactions between proteins and carbohydrates mediate many important cellular functions, such as protein folding, immune cell signaling, and viral entry into host cells. Despite the known importance of carbohydrate-protein interactions, the characterization of these interactions remains challenging. This challenge is ultimately due to the weak binding affinity of many carbohydrate-protein interactions, which typically exhibit low millimolar binding affinities. To circumvent this issue, our lab is developing new chemical tools capable of identifying interactions between carbohydrate-binding proteins and a carbohydrate of interest. These molecules utilize principles of ligand-directed chemistry, in which we can covalently label the carbohydrate-binding protein in a carbohydrate-directed manner despite the weak affinity of the interaction. These tools will ultimately be used to identify interactions between proteins and carbohydrates in living systems and characterize these interactions in disease relevant pathways. Here we describe our synthetic approach to these molecules and their initial biological characterization.

The Role of Mitochondrial-Microbial Interactions in Regulating Brain-Gut Health

Emily A. Beck

Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

Understanding how mitochondria (mito) and microbial (micro) interactions impact brain-gut health is important for the study of age-related disorders like Alzheimer's Disease (AD), Parkinson's disease, and ALS where mitochondrial dysfunction and brain-gut dysbiosis are common. In many cases, mitochondrial dysfunction leads to an overproduction of reactive oxygen species (ROS) which are naturally produced during energy production. At low levels ROS is important for cell signaling and at high levels causes oxidative stress and neuroinflammation. Elevated ROS can also impact resident microbes, reducing microbiome diversity, and causing gastrointestinal distress. These

mito-micro interactions are rooted in their shared evolutionary history; with mitochondria evolving from alphaproteobacteria during the early evolution of eukaryotic life. As such, mitochondria and bacteria have retained similar morphological features. These shared features provide shared targets for metabolites – like ROS – and therapeutics. One important example is antibiotics, commonly used to target bacteria. Antibiotics often target shard mito-micro morphological features including shared membrane components or shared machinery for protein synthesis. Chloramphenicol is an antibiotic known to negatively effect mitochondria by targeting shared features of bacterial protein synthesis machinery. In zebrafish, exposure to chloramphenicol leads to disrupted mitochondrial translation and function. Recently, exposure to chloramphenicol has also been shown to elevate genetic markers for AD in neuronal cell culture. We are exploring (1) if chloramphenicol can elevate AD genetic markers in vivo, (2) what chloramphenicol does to mito-micro homeostasis, and (3) if we can identify specific genetic variants leading to elevated mitochondrial dysfunction and brain-gut dysbiosis.

How Alterations in the Chemical Composition of Repaired Myelin Impacts its Biophysical Properties

Estephanie L. Nottar Escobara, Nishama D. S. Mohottib, Mara Manolescua, Anika Radadiyaa, **Prajnaparamita Dhar**[1], and Meredith D. Hartley[2]

[1]*Department of Chemical Engineering, University of Kansas, Lawrence, KS, USA;*
[2]*Department of Chemistry, University of Kansas, Lawrence, KS, USA*

The myelin sheath is a lipid-rich membrane that ensheathes axons and is required for healthy and efficient signal transduction. In neurological diseases like multiple sclerosis, the myelin is damaged. But remyelination can occur through the action of oligodendrocyte precursor cells (OPCs), which differentiate into mature oligodendrocytes that wrap axons to form repaired myelin. Here we will discuss how a genetic-based mouse model of demyelination, which features near-complete demyelination followed by robust remyelination in the brain, was used to study the chemical composition of the lipids in the myelin sheath. Changes in the chemical composition were compared and corelated with changes in the mechanical properties of the myelin sheath.

Lipid mass spectrometry on isolated myelin from the remyelinated brain revealed a decrease in the percent mole fraction of cholesterol when compared to healthy myelin. Biophysical studies on monomolecular lipid films formed using myelin lipid extracts from repaired myelin showed changes in the surface behavior of the lipid films, compared to the healthy myelin. Films formed using the remyelinated lipid extracts resulted in lower surface pressures and lower compressional moduli when compared to healthy controls, suggesting that repaired myelin membranes have lower lateral molecular packing within the lipid film. Synthetically prepared model membranes, based on the major lipid compositions of the healthy and diseased extracts, revealed that changes in cholesterol levels were the primary contributor to the changes in biophysical properties. Interestingly, altering the composition of the major phospholipid compositions did not show significant changes in the biophysical properties. Supplementation of the diseased lipid extracts with cholesterol led to a robust improvement in membrane surface pressures and compressibility.

Together, these results suggest that high cholesterol levels are required for myelin membrane stability and that reduced cholesterol in repaired myelin may have a profound impact on the biophysical properties of the myelin membrane.

Redefining the Role of AMPK in Autophagy Regulation

Carlo Barnaba

Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, USA

Autophagy is a critical metabolic pathway that recycles cellular components, either non-selectively or through the targeted degradation of protein aggregates and damaged organelles. Its process begins when autophagy factors assemble on ATG9-containing vesicles, forming phagophores. These phagophores expand by tethering to donor membranes and facilitate ATG2-mediated lipid transfer, eventually maturing into autophagosomes that fuse with lysosomes for degradation. While autophagy activation by amino acid starvation is well-established, its regulation under low energy conditions—mediated by the AMP-activated protein kinase (AMPK)—remains unclear. Using live-cell imaging, our lab is dissecting how AMPK regulation via glucose starvation or AMPK-targeting drugs regulates autophagosome biogenesis. We demonstrate that AMPK activation inhibits autophagosome maturation by suppressing AMPK-mediated phagophore tethering to donor membranes. These findings clarify AMPK's role in autophagy and redefine its potential as a therapeutic target for modulating autophagic activity.

Folding Outer Membrane Proteins into Catanionic Detergent Vesicles

Andrew Daufel[1], Alex Bowman[1], Jason T. Kaelber[3,4], Ashley Bernstein[3,4], Joanna S.G. Slusky[1,2]

[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; [2]Computational Biology Program, University of Kansas, Lawrence, KS, USA;[3]Rutgers Cryo EM & Nanoimaging Facility and Institute for Quantitative Biomedicine, Rutgers University, New Brunswick, NJ, USA;[4]Department of Chemistry and Chemical Biology, Rutgers University, New Brunswick, NJ, USA

The emergence of life required the separation of the self from an environment. This separation is accomplished through a membrane— an aggregation of amphipathic molecules forming a hydrophobic bilayer. What was the first membrane that could accommodate proteins? LUCA is thought to have many similarities to Gram negative bacteria including features of the Gram negative outer membrane. In Gram negative bacteria, the outer membrane is an asymmetric bilayer with negatively charged lipopolysaccharides on the outer leaflet and zwitterionic phospholipids on the inner leaflet. A feature of an asymmetrically charged membrane is that it can be created by single tail lipids that were available earlier in evolution than multi-tailed lipids like those in more modern phospholipid bilayers. We developed model charge-asymmetric membranes using single tail lipids and determined that proteins can fold into them. Our

asymmetrically-charged detergent vesicles offer an opportunity to consider how proteins became part of membranes in the proto-life environment and allow us to test the effects of charge asymmetry on membrane protein folding.

Poster Abstracts – Saturday, October 11th

Transcription and Regulation of the *mpt* PTS in *Enterococcus faecalis*

#1 - Tolulope I. Ade and Lynn E. Hancock

Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

Enterococcus faecalis is a metabolically versatile organism that has been reported to encode at least 46 different phosphotransferase systems (PTS). The PTS is a phospho-relay system that is important for the uptake and phosphorylation of carbon substrates for energy production. Six of the 46 PTSs in *E. faecalis* are regulated by the alternative sigma factor, σ54 (RpoN) that recognizes the -24/-12 promoter sequence. The regulation of RpoN-dependent PTSs also require bacterial enhancer binding proteins (bEBPs) that belong to the LevR family of transcriptional regulators and include CelR, DgaR, GfrR, MptR, and XpoR. Using biolog growth phenotype assay and sugar-specific growth phenotype assays, we have defined RpoN-dependent substrates that require CelR, DgaR, GfrR, and MptR for their uptake. We have also used luciferase assay to show that these sugars specifically induce the different promoters that are regulated by the respective bEBPs. Specifically, the *mpt* PTS, regulated by MptR, is important for the uptake of glucose, mannose, glucosamine, and N-acetylglucosamine. Bioinformatic analysis predicts that the *mpt* operon contains four open reading frames – *mptB* (EIIB), *mptA* (EIIA), *mptC* (EIIC), and *mptD* (EIID). More detailed analysis of this operon identified a second EIIB domain fused to the EIIA protein of MptA and shares ~ 50% amino acid sequence similarity with MptB. We hypothesize that the presence of two EIIB domains within the Mpt operon enables diversification of the sugar substrates recognized and are currently undertaking a genetic deletion approach to identify contributions from each EIIB domain.

#2 - Spectroscopic Properties and Reactivity of a Mn^{III}-hydroperoxo Complex that is Stable at Room Temperature

Zahra Aghaei, Elizabeth N. Grotemeyer, and Timothy A. Jackson

Department of Chemistry, University of Kansas, Lawrence, KS, USA

The manganese lipoxygenase enzyme (MnLOX) which converts polyunsaturated fatty acids to alkyl hydroperoxides is found in fungi and plants to allow further metabolization of these substrates. It is proposed that the substrate oxidation is initiated by the transfer of a hydrogen atom from the C-H bond of the substrate to an active-site Mn^{III}-hydroxo center. Inspired by these enzymes, there are many examples of synthesized model manganese complexes to study the active site of enzymes. These synthesized models were able to activate H_2O_2 to conduct a series of reactions such as sulfoxidation, olefin epoxidation and C-H bond activation with high selectivity. It is generally proposed that in both enzyme and synthesized Mn catalysts a Mn^{V}-oxo is the key intermediate which is formed from the O-O bond cleavage of a Mn^{III}-hydroperoxo. This Mn^{III}-hydroperoxo intermediate possesses critical role in Mn catalytic cycle but there are a few reports on them. In this study, we formed a Mn^{III}-hydroperoxo supported

by an amide-containing, pentadentate ligand ($dpaq^{6Me}$). The Mn^{III}-hydroxo complex $[Mn^{III}(OH)(^{6Me}dpaq)]^+$ reacted with H_2O_2 and $HClO_4$ to form a green intermediate, with a UV-vis band at 615 nm. On the basis of ^1H NMR, ESI-MS, EPR, FT-IR, and EPR data, we formulate this new intermediate as the Mn^{III}-hyrdroperoxo complex $[Mn^{III}(OOH)(^{6Me}dpaq)]^+$. This complex reacts with 10 equivalences of PPh_3 at 15 °C, and with 30 equivalences of TEMPOH at -35 °C. Based on kinetic studies, $[Mn^{III}(OOH)(^{6Me}dpaq)]^+$ reacts 600-fold faster with PPh_3 than its Mn^{III}-alkylperoxo analogue $[Mn^{III}(OO^tBu)(^{6Me}dpaq)]^+$, and two-fold faster with TEMPOH than a similar Mn^{III}-hydroperoxo complex $[Mn^{III}(OOH)(dpaq^{2Me})]^+$. Time dependent computations (TD-DFT) were applied to compare electronic features of $[Mn^{III}(OOH)(^{6Me}dpaq)]^+$ with analogous Mn^{III}-hydroperoxo and Mn^{III}-alkylperoxo complexes.

#3 - Boronic Acid - a Multifaceted Tool for Enhanced Identification of Cross-linked Peptides

Emmanuel Ajiboye, Mitali Sameer Thole, Hyma Mandapaka, Haifan Wu

Department of Chemistry and Biochemistry, Wichita State University, Wichita, KS, USA

In living organisms, almost all cellular functions rely on intricate three-dimensional protein machinery and protein-protein interactions (PPIs). To gain a better understanding of key biological processes, tremendous effort has been put into deciphering protein structures and mapping PPI networks. Cross-linking mass spectrometry (XL-MS) is a reliable approach that may offer conformational constraints inside proteins and protein complexes, allowing for the modeling of protein structures and analysis of PPI networks. Protein samples are chemically cross-linked in XL-MS, then digested by proteases and analyzed by LC-MS/MS to identify cross-linked peptides. However, one disadvantage of XL-MS is the complexity of the samples. It is challenging to identify cross-linked peptides in the mixture due to their low abundance. Here, we create a robust affinity purification (AP) process for enriching cross-linked peptides before LC-MS/MS analysis. Boronic acids (BAs) can form reversible covalent bonds with diols and are highly versatile as building blocks for a variety of useful chemical reactions. Furthermore, several BA-containing medications have been approved by the FDA, indicating that they are safe. As a result, we investigated how boronic acid could be used to enhance cross-linked peptides. We developed BA-containing cross-linkers and a diol-containing AP resin. Using model proteins, we discovered that BA-based enrichment is extremely sensitive and specific. As a result, we expect that this new enrichment handle will enhance XL-MS's ability to analyze complex protein materials.

#4 - Three *Chlamydia muridarum* Tn Mutants Exhibit Reduced Ascension to and Pathology in the Mouse Upper Genital Tract

Lauren Atkinson, Dominique Jaramillo, Scott Hefty

Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

Relatively few Chlamydia gene products have been directly assessed for their contribution to mammalian infection and pathology. In this study, fifteen transposon (Tn) mutants from a *C. muridarum* library were individually assessed for their ability to ascend to the upper reproductive tract. C57BL/6 mice were infected intravaginally, and vaginal vault and uterine horn tissues were harvested at 7 days post-infection. Bacterial burdens were determined using ddPCR to quantify chlamydial genome copies relative to host genomes for each tissue. Eleven mutants exhibited bacterial burdens similar to wild-type *C. muridarum* in both tissues. Four Tn mutants also exhibited bacterial burdens similar to wild-type in the vaginal vault but significantly reduced burdens in the uterine horns ($p<0.05$; Welch's t-test). The four mutants with an ascension defect were further assessed for pathogenicity in a chronic infection model. Three mutants displayed reduced hydrosalpinx at 56 days post-infection ($p<0.05$; Mann-Whitney U test). The Tn mutants defective for both uterine horn ascension and pathology are TC0350 (*folD* CT078), TC0530 (*cppA* CT259), and TC0075 (CT702). Both *cppA*::Tn and TC0075::Tn exhibited significant *in vitro* growth defects, while *folD*::Tn exhibited normal *in vitro* growth, suggesting that this disruption has a stronger effect on *in vivo* infection functions. The growth defect for *cppA*::Tn, a phosphatase involved in early differentiation events, was predicted based on recent studies, but the function of the conserved chlamydial-specific hypothetical protein TC0075 is unknown. These results suggest that it may play a crucial role in the basic biology of Chlamydia. These observations link the role of an enzyme involved in tetrahydrofolate metabolism to both the key virulence step of upper genital tract ascension and pathology.

#5 - Non-canonical Autophagy Induction by Proanthocyanidins in Human Cancer Cells

Oluwabukunmi Ayotunde Babalola and Carlo Barnaba

Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, USA

Cancer and aging are intricately linked to dysfunctional autophagy and mitophagy, cellular processes essential for maintaining homeostasis and mitigating oxidative stress. Diet plays a critical role in promoting resilience and healthy aging; however, establishing direct links between dietary components and health outcomes remains challenging due to limited mechanistic understanding at the cellular level. Investigating polyphenol-derived small molecules, such as polymeric proanthocyanidins (PAs), offers a promising avenue for uncovering mechanisms that regulate autophagy and mitochondrial stress in cancer.

Recent studies have recognized polyphenols for their antioxidant, antiproliferative, and antisenescence effects. To date, few studies have examined how PAs mechanistically modulate autophagy within the context of cancer.

Here, we show that PA treatment of U2OS osteosarcoma cells reduces proliferation and increases LC3-II accumulation, indicating autophagy induction. Live-cell imaging with endogenously Halo-tagged ATG13 expressing GFP-LC3 revealed robust

LC3 puncta formation and recruitment without colocalization with ATG13, suggesting that PA-induced autophagy bypasses the canonical ULK1-ATG13 pathways. Furthermore, resveratrol – a monomeric polyphenol – also induced non-canonical LC3 accumulation, pointing to a shared mechanism among structurally related polyphenols.

These findings identify PAs as modulators of alternative, non-canonical autophagy mechanisms, providing insight into defining their chemotherapeutic targets for cancer therapy and healthy aging.

#6 - Potentiostat-free Electrochemiluminescence Reporting for Microchip Electrophoresis-based Detection of Nitrotyrosine-containing Peptides

Soum Barua[1,2,3], Indika Warnakula[3,4], Susan M. Lunte[1,2,3,4]

[1]Department of Chemistry, University of Kansas, Lawrence KS, USA; [2]The Ralph N. Adams Institute for Bioanalytical Chemistry, University of Kansas, Lawrence, KS, USA; [3]Department of Petroleum and Chemical Engineering, University of Kansas, Lawrence, KS, USA; [4]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence KS, USA

Reactive nitrogen and oxygen species (RNOS) are generated by cells during oxidative stress, a process involved in neurodegenerative disorders, including Alzheimer's disease. Tyrosine (Y) can react with peroxynitrite, an RNOS product of nitric oxide and superoxide, to generate nitrotyrosine (NY), which can act as an *in vivo* biomarker of oxidative damage. Mass spectrometry is most often used for the determination of nitrotyrosine-containing proteins, but it is both large and expensive. In this paper, a method for determining NY using microchip electrophoresis with potentiostat-free electrochemical reduction of nitrated peptides at a bipolar electrode, coupled with optical electrochemiluminescence (ECL), is presented. The current system consists of a simple-t microchip for the electrophoretic separation and a separate straight reporter channel for the ECL. A bipolar electrode connects the two channels. Voltages applied across the separation and reporter channels generated an electrophoretic separation of analytes and electroosmotic flow or the reporter probe solution. It also sets the potential of the bipolar electrode. Electrochemiluminescence was achieved in the reporter channel using luminol/H_2O_2 and measured with a PMT. Optimization of the device for the selective detection of nitrotyrosine-containing peptides will be described.

Keywords: microchip electrophoresis, bipolar electrode, limits of detection, electrochemiluminescence detection

#7 - Inflammation and Epigenetic Priming in Zebrafish Induced by Early-Life Hypoxia: A Developmental Basis for Cardiovascular Disease

Syeda Kishuara Begum[1] and Allie Graham[1,2]

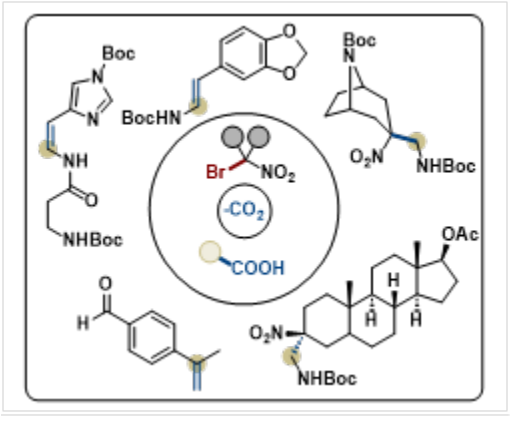
[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; [2]KU Center for Genomics, University of Kansas, Lawrence, KS, USA

Hypoxia (low oxygen) acts as a stressor in the progression of cardiovascular diseases (CVDs) with long-term consequences on ischemic heart disease, heart failure, pulmonary hypertension, and stroke. Although developmental exposure to hypoxia can permanently alter vascular and inflammatory pathways, it is still unclear how early hypoxia predisposes individuals to CVD in later life. One of the main mediators of hypoxia-induced damage is inflammation, although it is unknown how immune transcriptional states evolve and remain stable in the early stages of development. Using hypoxia-tolerant vertebrate model zebrafish (*Danio rerio*), this study investigates how developmental hypoxia influences immune activation and epigenetic remodeling, related to the progression of CVD. Our preliminary evolutionary genomic analyses revealed that a number of hypoxia-associated genes under positive or purifying selection in high-altitude vertebrates are abundant in inflammatory pathways. In Aim 1, I will test how developmental hypoxia activates pro-inflammatory transcriptional responses in macrophages and neutrophils by FACS-sorting immune cells exposed to graded hypoxia and utilize bulk RNA-seq to assess cytokine expression. In Aim 2, I will test epigenetic modifications and chromatin accessibility by performing ATAC-seq and CUT&RUN, and assess vascular remodeling through confocal imaging of transgenic zebrafish. Together, these goals will shed light on how early hypoxia reprograms immune transcription and chromatin states to induce long-term CVD susceptibility. These questions form the basis of a proposal that I am writing for the AHA predoctoral fellowship.

#8 - Leveraging Decarboxylative Transformations to Develop Sustainable Synthetic Methods for Bioactive Molecules Using *gem*-Bromonitroalkanes

Deshkanwar Singh Brar, Ebbin Joseph, Roberto Aponte, Jon A. Tunge

Department of Chemistry, University of Kansas, Lawrence, KS, USA



Building a sustainable future necessitates reducing our reliance on fossil fuel-derived feedstocks for the synthesis of pharmaceuticals. Biomass-derived substrates, particularly carboxylic acids, have emerged

as promising alternatives. With improvements in extraction technologies, these shelf-stable carbon building blocks, such as amino acids, fatty acids, and sugar acids, have become more accessible. Our research shows that thermal decarboxylation of these eco-friendly molecules provides a sustainable and cost-effective route to styrenes, which are precursors used in the synthesis of various therapeutics. Mechanistically, this transformation proceeds via thermal decarboxylation followed by halogen-ion transfer from readily available *gem*-bromonitroalkanes (BNAs). In addition, we adapted this halogen transposition to occur through free-radical intermediates. This enabled the valorization of amino acids and dipeptides, including Nateglinide, L-DOPA, carnosine, and aspartame, into enamides, which constitute a key structural motif in many bioactive molecules. Furthermore, this strategy facilitated the conversion of alkaloids and steroids, such as nortropinone and androstanolone, to β-nitroamines without the need for transition metals. Overall, our results show that the merger of decarboxylation with intermolecular halogen transposition provides a mild, green, and efficient approach to the synthesis of diverse medicinally-relevant molecules.

#9 - VE-cadherin Peptide Fragments as Modulators of the Blood-Brain Barrier: Artificial Intelligence and Machine Learning

Clayton Broockerd[1], Teruna J. Siahaan[2], Krzysztof Kuczera[1,3]

[1]Department of Molecular Biosciences, The University of Kansas, Lawrence, KS, USA; [2]Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS, USA; [3]Department of Chemistry, The University of Kansas, Lawrence, KS

Interactions between cadherin proteins are a crucial aspect of the *adherens* junction, a part of the Blood-Brain Barrier, which hinders delivery of therapeutic agents from the bloodstream to the brain. Previously, cadherin-derived peptides in the HAV and ADT families were developed in our group as effective BBB modulators, exhibiting cadherin binding, inducing BBB permeability and allowing brain delivery of large molecules, including antibodies. Their mechanism of action involves disrupting zipper-like interactions between the first two cadherin extracellular domains, EC1 and EC2. To explore a wider range of BBB modulators, we model the interactions of all consecutive 6-residue fragments of EC1 and EC2 of human VE-cadherin with the parent protein. First, the AlphaFold3 Artificial Intelligence model was used to predict the 3D structures of the peptide-protein complexes from sequences. Next, the binding affinities of the model complexes were estimated with the PRODIGY Machine Learning model. Out of the ~200 studied 6aa sequences, several exhibited higher predicted VE-cadherin binding affinities than previously investigated peptides. After experimental confirmation of binding potency, BBB modulating efficacy and pharmacological characterization, these peptides could be developed as therapeutic agents to aid in delivery of drugs and diagnostics to the brain, potentially contributing to curing neurodegenerative diseases, such as Alzheimer's disease.

#10 - Improved Transformation Efficiency of Chlamydia using Transferrin Delivery

Sofía Chacón-Araya, Dominique Jaramillo, P. Scott Hefty

Department of Molecular Bioscience, University of Kansas, Lawrence, KS, USA

The transformation of *Chlamydia* is an important tool in identifying genes essential to infection. *Chlamydia muridarum* is used as a model organism of the human strain, *Chlamydia trachomatis*, in the animal model studies in mice. In order to have successful results, *C. muridarum* transformations need to improve since they have a lot more limitations when it comes to transformations. Based on previous publications there might be a way to optimize those transformations by using transferrin as a DNA carrier.

#11 - Efficacy of Antibiotic Prophylaxis for Infective Endocarditis Recommended for Dental Patients

Gabriela C. Chianca[1,2,3], Helvécio C. C. Póvoa[1], Bruna A. Thurler[1,2], Raiane C. Chamon[2], Fábio F. da Mota[4], Rosana B. R. Ferreira[3], Natalia L. P. P. Iorio[1].

[1]Department of Basic Sciences, Universidade Federal Fluminense (UFF), Nova Friburgo, RJ, BRA; [2]Department of Pathology, UFF, Niterói, RJ, BRA; [3]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; [4]Department of Systems and Computational Biology, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, BRA.

Infective endocarditis (IE) is an infection caused by microorganisms that reach the bloodstream and cardiac structures. Within the heart, they form vegetations (biofilm-like structures) that can cause direct damage to the heart tissue and/or fragment and reach other organs. To prevent the development of IE associated with invasive dental procedures, clinical guidelines recommend the use of antibiotic prophylaxis to prevent IE caused by oral *Streptococcus* on patients at high risk for IE. However, its efficacy is still debated. This study aimed to define the susceptibility profile of oral microorganisms isolated from IE belonging to the *Streptococcus* genus isolated between 1935-2021 from 12 countries and evaluate the ability of some of these species to produce biofilm in the presence of IE prophylactic antibiotics. Furthermore, we aim to evaluate the impact of metabolites produced by skin microbiome members with previously shown antibiofilm activity on *Streptococcus* biofilm formation. To do this, first we have conducted an *in silico* study to understand the susceptibility profile of *Streptococcus* isolated from patients with IE, whose assemblies were sequenced and present in the RefSeq database, and to correlate this profile with the dental prophylaxis recommended for IE. Then, we plan to analyze biofilm formation of *Streptococcus sanguinis* and *Streptococcus gordonii* isolated from patients with IE, under different conditions, including in the presence of skin microbiome metabolites. We will also evaluate biofilm formation of these strains under continuous flow in the presence and absence of antibacterial agents used in IE prophylaxis. Our *in silico* analysis of 258 *Streptococcus* isolated from EI revealed that *Streptococcus oralis* was the most prevalent species. Ninety-nine (38.4%) of the 258 analyzed assemblies showed potential

resistance to at least one antimicrobial, and 34 were multidrug-resistant. Most genomes were from oral *Streptococci* (79.5%), and among these, 37.1% presented resistance genes to at least one antimicrobial. None of the genomes showed potential resistance to the antimicrobial used as primary prophylaxis for IE (amoxicillin), nor to those antimicrobials used as an alternative for patients unable to take oral medications (ampicillin, cefazolin, and ceftriaxone). However, resistance genes for azithromycin and/or doxycycline (two of the six antimicrobials currently recommended as alternatives for patients allergic to penicillin/ampicillin) were observed in all 76 genomes of oral samples that were resistant to at least one antimicrobial. Analysis of biofilm formation of *S. sanguinis* and *S. gordonii* revealed that BHI supplemented with 1% sucrose was the optimal condition to evaluate biofilm formation. Defining the best media for biofilm formation will allow us to provide the best conditions to test for antibiofilm activity of the metabolites as well as evaluate the impact of antibiotics on biofilm formation under continuous flow models, which better mimic *in vivo* conditions.

Keywords: Microbiology, Endocarditis, Dentistry, Disease Prevention, Biofilm.

#12 - Incorporation of Chimeric Macrodmains into Murine Hepatitis Virus for Antiviral Testing of Multiple Coronaviruses in a Single System

Kendall A. Cranor, Anjali Singh, Nathan Quinton, Jessica J. Pfannanstiel, Anthony R. Fehr

Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

The Macrodomain 1 (Mac1) protein is a critical virulence factor of coronaviruses (CoVs) that promotes viral replication, suppresses the host immune response, and is required for disease in animal models. Mac1 both binds and enzymatically removes ADP-ribose, a post-translational modification, from target proteins, allowing the virus to evade the host innate immune response. Deletion of Mac1 from the CoV genome leads to a significant defect in viral replication and rescues host immune activity, indicating that Mac1 inhibitors could be effective against CoVs. To characterize the genetic variation of Mac1 proteins, we will insert Mac1 genes of diverse CoVs into murine hepatitis virus (MHV), in place of the native MHV Mac1 protein. To do this, we will clone Mac1 genes into the MHV genome in *E. coli* by Lambda Red Recombination, a genetic engineering technique that uses bacteriophage lambda proteins to facilitate homologous recombination. We replaced the the Mac1 gene of MHV, a β-CoV, with Mac1 from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), another β-CoV. Surprisingly, this virus replicates just like the original MHV, indicating a close evolutionary relationship of their Mac1 proteins. This system will be applied to the Mac1 genes from another β-CoV, Middle East respiratory syndrome virus (MERS-CoV), an α-CoV, porcine epidemic diarrhea virus (PEDV), a γ-CoV, infectious bronchitis virus (IBV), and a δ-CoV, porcine delta coronavirus (PD-CoV). This could provide insight into the evolution and genetic diversity of Mac1 proteins across the CoV genera and facilitate the testing of antivirals targeting Mac1 of multiple CoVs.

#13 - Folding Outer Membrane Proteins into Catanionic Detergent Vesicles

Andrew Daufel[1], Alex Bowman[1], Jason T. Kaelber[3,4], Ashley Bernstein[3,4], Joanna S.G. Slusky[1,2]

[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; [2]Computational Biology Program, University of Kansas, Lawrence, KS, USA;[3]Rutgers Cryo EM & Nanoimaging Facility and Institute for Quantitative Biomedicine, Rutgers University, New Brunswick, NJ, USA;[4]Department of Chemistry and Chemical Biology, Rutgers University, New Brunswick, NJ, USA

The emergence of life required the separation of the self from an environment. This separation is accomplished through a membrane— an aggregation of amphipathic molecules forming a hydrophobic bilayer. What was the first membrane that could accommodate proteins? LUCA is thought to have many similarities to Gram negative bacteria including features of the Gram negative outer membrane. In Gram negative bacteria, the outer membrane is an asymmetric bilayer with negatively charged lipopolysaccharides on the outer leaflet and zwitterionic phospholipids on the inner leaflet. A feature of an asymmetrically charged membrane is that it can be created by single tail lipids that were available earlier in evolution than multi-tailed lipids like those in more modern phospholipid bilayers. We developed model charge-asymmetric membranes using single tail lipids and determined that proteins can fold into them. Our asymmetrically-charged detergent vesicles offer an opportunity to consider how proteins became part of membranes in the proto-life environment and allow us to test the effects of charge asymmetry on membrane protein folding.

#14 - Optimizing the Delivery of Antibody Therapeutics using Blood Brain Barrier Modulatory Peptides

Eric Ebert[1], Farid Hajareh Haghighi[1], Kelly Schwinghamer[1], Vinith Yathindranath[2], Donald W. Miller[2], Teruna J. Siahaan[1]

[1]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, United States [2]Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, MB, Canada

Monoclonal antibodies (mAbs) are an important class of therapeutics to potentially treat a wide variety of brain diseases such as Alzheimer's disease (AD), multiple sclerosis, glioblastoma, and other brain cancers. Due to their physicochemical properties, mAb therapeutics cannot readily diffuse across the blood-brain barrier (BBB) into the brain. One way to improve the delivery of mAbs across the BBB is via the paracellular pathway of the BBB. Because the BBB *adherens* junction is mediated by VE-cadherin interactions that seal the paracellular space, modulation of cadherin-cadherin interactions with cadherin peptides (i.e., ADTC5, HAVN1) as BBB modulators (BBBMs) can increase the paracellular porosity of the BBB to allow the permeation of various therapeutic molecules into the brain. Previously, BBBMs enhanced the BBB penetration of a wide

variety of molecules into the brain including ^{14}C-mannitol, gadopentetic acid (a magnetic resonance imaging contrast reagent), 13.5 kDa brain derived neurotrophic factor (BDNF), 25 kDa polyethylene glycol, 65 kDa albumin and 150 kDa mAbs. In this project, our goals are to (1) optimize the delivery of mAbs to the brain using newly developed BBBMs from VE-cadherin; (2) evaluate the safety of repeated use of BBBMs *in vivo*; (3) evaluate the efficacy of BBBMs when administered through the carotid artery; and (4) study the binding properties of BBBMs to VE-cadherin. Recently, two new BBBM peptides were found from the sequence of VE-cadherin and these peptides increased the brain deposition of a mAb. Multiple injections of several BBBM peptides indicated that the BBBM peptides were not toxic in mice. Carotid artery administration of BBBMs with mAbs led to a 20-fold increase of mAb levels in the targeted hemisphere. Preliminary data also suggest that the BBBMs can deliver lipid nanoparticles to the brain through the carotid artery. In the future, the BBBMs will be used to enhance the brain delivery of BDNF or BDNF peptides in a mouse model for AD.

#15 - Defining How Different Sulfation Patterns in the Extracellular Matrix Impact Myelin Repair in the Brain.

Corbin Fairchild, Jenna Williams, Matthew Zupan, Dr. Esther Holt, Jack Petersen, Meredith Hartley

Department of Chemistry, University of Kansas, Lawrence KS, USA

Multiple sclerosis is a
neurodegenerative disease characterized by autoimmune degradation of myelin, known as demyelination. Myelin is a lipid-rich sheath produced by oligodendrocytes, which coats the axons of nerve cells and allow actions potentials to travel effectively. For successful myelination to occur, oligodendrocyte progenitor cells (OPCs) must differentiate into mature oligodendrocytes. This process can be affected by many factors; previous studies have shown that chondroitin sulfate proteoglycans (CSPGs), a major constituent of the extracellular matrix, play an important role in the development of nerve cells and maintenance of the nervous system.

CSPGs contain a protein core with chondroitin sulfate (CS) glycosaminoglycan (GAG) disaccharide chains. Different sulfonation patterns of the GAG chains influence their function. Our lab has previously demonstrated that the CS-E motif can inhibit OPC differentiation and remyelination. To study how CS-E impacts demyelination and remyelination, we will induce demyelination in a global knockout of carbohydrate sulfotransferase 15 (Chst15), the enzyme that converts CS-A to CS-E. We will administer cuprizone compounded into the to induce demyelination and study the effect of myelination, oligodendrocytes, and OPCs will be examined. Our goal is to elucidate the function of Chst15 in OPC differentiation by quantifying the number of new oligodendrocytes and OPCs in our model. To quantify the number of new

oligodendrocytes and OPCs, EdU will be administered in the water of mice to label newly forming cells. We predict that mice lacking CSE will have improved remyelination and increased OPC differentiation.

#16 - AlphaFold3 Model of *Shigella* Translocon Proteins

J. Tucker Folscroft and Roberto N. De Guzman

Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

Many Gram-negative pathogens assemble the type III secretion system injectisome to inject virulence proteins into host cells to cause infectious diseases. Part of the injectisome is the translocon pore on the host membrane to allow the passage of virulence proteins directly into the host. In *Shigella*, IpaB and IpaC are the translocase proteins and IpaD is the tip protein. IpaB interacts with IpaC to form the translocon, which is docked on the IpaD tip complex. IpaB is predicted to contain 2 transmembrane (TM) helices, while IpaC is predicted to have 1 TM helix. The atomic structure of IpaB in a membrane and how it interacts with IpaC and IpaD are unknown. The only known atomic structure for IpaB (580-residues) is its N-terminal domain (residues 7-242). We used AlphaFold3 to model the atomic structure of IpaB in a membrane, and its interaction with IpaC and IpaD. AlphaFold3 suggested that the IpaB N-terminal domain and C-terminal domain interact with each other. AlphaFold3 also suggested possible interaction of the transmembrane helices of IpaB and IpaC. These models allow us to design probes to test the atomic model by fluorescence and NMR spectroscopies.

#17 - Novel Toll-Like Receptor 7/8 Antagonist Promotes IL-10 Mediated Anti-inflammatory Therapy

Grahmm A. Funk[1], Jin Xie[1], Tamara A. Kucaba[2], Thomas S. Griffith[2], David M. Ferguson[3], Hyunjoon Kim[1]

[1]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, USA; *[2]Department of Urology, University of Minnesota, Minneapolis, MN, USA;* *[3]Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN, USA*

Purpose: Toll-like receptors (TLR) 7 and 8 are pattern recognition receptors expressed in immune cells that respond to viral and bacterial infections. TLR7/8 activation triggers a pro-inflammatory immune cascade that leads to T cell and NK cell activation. Hence, synthetic imidazoquinoline-structured TLR7/8 agonists were developed as immunotherapy candidates for cancer and as infectious disease vaccine adjuvants. However, whether antagonizing TLR7/8 can induce the opposite effect, which is to produce anti-inflammatory cytokines and induce immunosuppressive cellular phenotypes, is a gap in knowledge. In this study, we investigated the immunosuppressive efficacy of a

novel TLR7/8 antagonist (621) using cellular and murine models of inflammation and compared the effects directly to a novel TLR7/8 agonist (558).

Methods: Bone marrow-derived dentritic cells (BMDCs) were harvested from C57BL/6 mice to assess 558 and 621 activation of DCs *in vitro*. Pro- and anti-inflammatory cytokine responses from BMDCs were measured via enzyme-linked immunosporbant assays (ELISAs) after pre-treatment with 558 or 621 followed by TLR stimulation using TLR4 agonist lipopolysaccharide (LPS) and canonical TLR7/8 agonist resiquimod (RESQ). A similar *in vivo* study was performed where mice received intraperitoneal (IP) injections of 558 or 621 followed by IP injections of LPS or RESQ. Serum cytokine levels were measured and splenic CD4+ T cell and macrophage co-stimulatory marker expression was assessed via flow cytometry. Finally, we employed the dextran sodium sulfate (DSS)-induced colitis model to determine whether IP dosing of 621 can mitigate inflammatory disease progression.

Results: 621 was not shown increase DC co-stimulatory marker expression, but was a potent inducer of IL-10 without triggering TNF-a production. Systemic administration of 621 led to increased serum IL-10 levels and increased the frequency of regulatory T cells, M2 macrophages, and B cells when challenged with other TLR agonists. Futhur, 621 systemic therapy mitigated DSS-induced inflammatory diesase progression by reducing colon pro-inflammatory cytokines and increasing splenic regulatory T cells.

Conclusions: Comparing the efficacy of TLR7/8 agonism to antagonism gave insight into how TLR activity may attenuate inflammatory stimuli from a variety of sources. Chiefly, TLR7/8 antagomism by 621 led to an increase in systemic anti-inflammatory B cell, T cell, and macrophage phenotypes in both TLR- and DSS-induced models of inflammation. Futhur, 621 was shown to prevent the release of inflammatory cytokines following TLR agonism *in vitro* and *in vivo*. In all, TLR7/8 antagonism may serve as a desirable modalilty for anti-inflammatory therapies.

Acknowledgements: This work was supported in part by a grant from CoBRE-Chemical Biology of Infectious Disease (NIH P20GM113117) (H.K) and by the Madison and Lila Self Graduate Fellowship (G.A.F).

#18 - Hyaluronic-Acid Based Immunotherapy for Lyme Disease

Julia Galvez[1,2], Jacob Edholm[2], Catherine Kerr[2], Michael Brandon[2], Erik Naylor[2], Dwight Deay[2], Scott Hefty[1], Peter Petillo[2]

[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA;
[2]Design Zyme LLC, Lawrence, KU Innovation Park, KS, USA

Immunotherapy remains one of the most effective and cost-efficient strategies for disease prevention and outbreak control. Advances in vaccine design over recent years have greatly reduced the risk of triggering autoimmune or allergic responses. Among these approaches, subunit peptide vaccines offer the advantage of inducing highly targeted

immune responses; however, they typically require adjuvants to enhance peptide stability, distribution, and immunogenicity. Current adjuvants are limited by side effects such as local inflammation, swelling, and allergic reactions, highlighting the need for safer alternatives. Carbohydrate-based adjuvants, particularly covalently bound systems, have gained attention in vaccine development. Hyaluronic acid (HA), with its well-established clinical safety profile, is especially promising as both an immunostimulatory agent and a delivery platform. In this work, we report the development of HA–peptide conjugates for a Lyme disease vaccine. Using a nanoparticle carrier, we prepare formulations with varying HA–peptide loadings and investigated the influence of HA molecular weight on immunogenicity against the disease's main pathogen, *Borrelia burgdorferi*. Conjugation was achieved via copper-catalyzed azide–alkyne cycloaddition, followed by nanoparticle assembly at different loadings. The resulting vaccine preparations will be tested in mice, and immune responses evaluated by ELISA. We hope our findings show that HA–peptide conjugates represent a safe and effective strategy for the development of next-generation Lyme disease vaccines.

#19 - University of Kansas Nanofabrication Facility: Equipment and Services

Ryan Grigsby[1,2] and Susan M. Lunte[2,3,4,5]

[1]The KU Office of Research, University of Kansas, Lawrence, KS, USA; [2]The Center for Molecular Analysis of Disease Pathways, University of Kansas, Lawrence, KS, USA; [3]The Ralph N. Adams Institute for Bioanalytical Chemistry, University of Kansas, Lawrence, KS, USA; [4]Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS, USA; [5]Department of Chemistry, University of Kansas, The University of Kansas, Lawrence, KS, USA

The Kansas University Nanofabrication Facility (KUNF) is a Core Lab supported by the KU Office of Research and the Center for Molecular Analysis of Disease Pathways COBRE. The KUNF primarily caters to researchers who are manufacturing micro- and nanofluidic devices for biomedical research, but has the equipment and resources to accommodate broad research applications with micro- and nanofabrication needs. The core lab consists of about 1,300 ft^2 of ISO class 5, 1,700 ft^2 of ISO class 6 and 1,250 ft^2 of ISO class 7 cleanroom space, housing tools and materials for techniques including photolithography, nano-imprint lithography, plasma (dry) etching (ICP-RIE), wet etching, thin film deposition, scanning electron microscopy (VP-SEM), atomic force microscopy, contact angle goniometry, ellipsometry, profilometry, wafer dicing, wire bonding, laser ablation and engraving, 3D printing, hot embossing, and COMSOL software for device modeling. In addition, the facility has numerous microscopes for general inspection, ovens and furnaces, ultrapure water, and dedicated process fume.

This facility is under the leadership of Dr. Susan Lunte, and the direction of Ryan Grigsby. Services and usage of the facility are available to researchers from all Kansas universities. Training is provided to new investigators and graduate students in the use of micro- and nanofabrication procedures and equipment. In addition, researchers from both non-Kansas academic and private industry institutions may contract with the facility for

consultation and services. Hourly and per-use rates apply for facility access, equipment usage, and staff labor. Consultation is free.

#20 - *Chlamydia trachomatis* Hypothetical Proteome Annotation, a Structure-based Deep Learning Pipeline.

Moises Gualapuro[1], Kevin Hybiske[2], Scott Hefty[3]

[1]Department of Computational Biology, University of Kansas, Lawrence, KS, USA; [2]Department of Medicine, University of Washington, Seattle, WA, USA; [3]Department of Molecular Biology, University of Kansas, Lawrence, KS, USA

The functional annotation of the *Chlamydia trachomatis* genome is hindered by its obligate intracellular lifestyle, which limits experimental accessibility and leaves nearly one-third of its proteome classified as hypothetical. To address this gap, we performed a comprehensive structural and functional analysis of 209 previously uncharacterized proteins. Using a state-of-the-art deep-learning pipeline that integrates AlphaFold3 for 3D structure prediction, followed by DeepFRI for structure-based functional inference, we generated reliable structural models and annotations. Of the 209 models, 37 (17.7%) showed very high confidence in both global and local structure, while 110 (52.6%) exhibited accurate global folds and consistent local features. Low confidence structures were analyzed for disorder propensity by LoRA-DR, followed by ModiDB for annotating. These 147 high-quality models enabled functional annotation via DeepFRI, assigning Gene Ontology (GO) terms using a confidence threshold greater than 0.5. Biological process terms were predicted for 147 proteins, molecular functions for 116, and cellular components for 144, with 112 proteins receiving annotations across all three categories. Additional 23 disordered proteins were annotated for function alone. Our findings reduced the proportion of hypothetical proteins in the *C. trachomatis* proteome from approximately 33% to just over 8%. Further analysis suggests that many of these proteins may participate in transport, amino acid and nucleic acid metabolism, energy production, structure and expression regulation. These findings offer new insights into chlamydial biology and enhance the functional landscape of *C. trachomatis*, contributing a structurally validated resource to guide future protein-protein interaction studies, experimental validation, and therapeutic exploration.

#21 - AI Approach to *Chlamydia Trachomatis'* Rsb System Interactions

Steven Conejo Gutiérrez, Lexie Cutter, Hefty P. Scott

Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

Chlamydia trachomatis (Ct) is most common bacterial sexually transmitted infection and the leading cause of preventable blindness worldwide. Ct is an obligate intracellular bacterial pathogen with a biphasic life cycle that alternates between an elementary body (EB), an infectious though metabolically inert form, and a reticulate body (RB), a metabolically active but noninfectious form. Even though the two stages are well documented in academic literature, the interconversion between

them remains largely unresolved. To further inquire into EB-RB transitions, this project is centered on studying the Regulator of sigma B (Rsb) transduction pathway. The Rsb pathway is a signal transduction pathway described originally in *Bacillus subtilis* in charge of the regulation of sigma factors. Core components of Rsb in Ct include the sensor phosphatase RsbU, the anti-anti-sigma factor RsbV1, the anti-sigma factor switch kinase RsbW, and an unidentified sigma factor target. These components interact between each other through phosphorylation or dephosphorylation activity to react to outside stress signal. To evaluate the Rsb protein relations, this project uses protein-protein and protein-ligand interactions analysis to assess structural determinants on the pathway regulation. This method has identified potential key residues mediating RsbW kinase and RsbU phosphatase activity employing machine learning approaches to protein structure and interfaces. In addition, homology- and ligand-based analyses were performed to compare RsbU with related chlamydial proteins, providing structural validation and a mechanistic explanation for its experimentally observed regulation. Collectively, these results advance mechanistic understanding of the Rsb signaling cascade in Ct, providing a structural framework for future biochemical validation and for dissecting its role in regulating developmental transitions that underline pathogenesis.

#22 - Next Generation Sequencing at KU Genome Sequencing Core

Jennifer L. Hackett[1,2,3], Kristen M. Cloud-Richardson [1,2,3], Erik A. Lundquist [1,2,3], Susan M. Lunte [1,4,5]

[1]Center for Molecular Analysis of Disease Pathways, University of Kansas, Lawrence, KS, USA; [2]Genome Sequencing Core, University of Kansas, Lawrence, KS, USA; [3]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; [4]Department of Chemistry, University of Kansas, Lawrence, KS, USA; [5]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence KS, USA

The Genome Sequencing Core (GSC) is one of three research service core labs in the NIH COBRE Center for Molecular Analysis of Disease Pathways (CMADP) at the University of Kansas (KU). The major mission of the GSC is to provide researchers with next-generation sequencing (NGS) technologies. NGS, carried out in a massively parallel fashion, has been revolutionizing bio-medical research and used in a growing list of applications. Projects supported by the GSC include de novo genome assembly, genome re-sequencing for identification of mutations and polymorphisms, transcriptome analysis (RNA-seq), and epigenomic and gene regulation studies such as ChIP-seq, Methyl-seq, and small RNA analysis. The GSC enhances the genomics infrastructure at KU by providing a range of Illumina sequencing platforms including the NextSeq2000 and NextSeq550 (mid-sized genome re-sequencing or transcriptome projects) and the MiSeq (metagenomic or targeted amplicon sequencing projects) to researchers at KU-Lawrence and across the region. To capture the full power of NGS, we provide a range of project support, including project consultation, sample quality check, sequencing library construction, Illumina sequencing, and FASTQ generation and demultiplexing. For latest

pricing, current sequencing queue, or other information, visit the Genome Sequencing Core's website: https://gsc.ku.edu/.

#23 - Ultra-High-Throughput Virtual Screening and Binding Site Prediction

Andrew M. Johannesen, David K. Johnson

Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

Modern protein structure prediction alongside an expanding chemical space of synthesizable druglike molecules necessitates computational methods for predicting activity between structure predictions and very large chemical libraries. Working with previously developed methods for finding druggable regions of proteins, we introduce a method for predicting activity at these regions across tens of billions of possible candidates. Using SMILES embedded vectors and GPU accelerated neural networks, we can dramatically enrich large datasets with little computational overhead. The networks are trained on ROCS similarity scores between pocket-defined exemplars and a selected training set, with a final ROCS search and ML classifier used to discard false-positives to distill down choice candidates for predicted activity against found druggable pockets.

#24 - Computational Chemical Biology Core: A Chemical Biology of Infectious Disease COBRE Core Laboratory

David K. Johnson

Computational Chemical Biology Core, University of Kansas, Lawrence, KS, USA

Part of the Chemical Biology of Infectious Disease COBRE at the University of Kansas, the Computational Chemical Biology Core (CCB) works in collaboration with the Molecular Graphics and Modeling (MGM) Laboratory to provide the computational resources and expertise to enhance the productivity of researchers studying infectious diseases, in addition to other projects. The CCB has the tools and expertise to perform virtual screening, small molecule docking, cheminformatics analysis of high-throughput screening hits, binding site prediction, protein/peptide/antibody modeling and docking (including Alphafold modeling), protein design, and molecular dynamics simulations.

We present fourteen vignettes of publications that were enhanced by collaboration with the CCB/MGM. Recent highlights include the identification inhibitors of ACMS decarboxylase and degraders of DNAJA1 via virtual screening, using modeling to identify the functional activity of Legionella pneumophila effector protein SidI, using modelling to assess the structural impact of clinically relevant point mutations of TRIM32, modeling the interaction between the Type III secretion system basal body and sorting platform proteins SctK and SctD from Pseudomonas aeruginosa, and the optimization of an inhibitor of PTPRD.

With the software and expertise to perform virtual screening, protein-small molecule docking, protein/peptide modeling/docking/design, and cheminformatic analysis, the

CCB is a valuable resource to enhance the productivity of biochemistry, biology, medicinal chemistry, and pharmaceutical chemistry researchers.

The CBID COBRE is funded by the NIH NIGMS grant 1P20GM113117.

The CCB works in collaboration with the Molecular Graphics and Modeling Laboratory.

#25 - Transfer RNA Fragment Analog as a Potential Therapeutic in ALS/FTD

Prakash Kharel[1], Pavel Ivanov[2], Paul Anderson[2]

[1]Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS, USA; [2]Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston MA, USA

Deregulated translation initiation is a hallmark of various cancers and neurodegenerative disorders, making it a compelling target for therapeutic intervention. We previously demonstrated that select tRNA-derived stress-induced small RNAs (tiRNAs) can repress translation initiation by interacting with the scaffolding protein Eukaryotic translation Initiation Factor 4G 1 (EIF4G1). Building on this foundation, we have engineered a series of tiRNA analogs and identified one candidate, TD-1, with exceptional translational repression capacity—exceeding that of native 5'tiRNA^{Ala} by over 1000-fold. Mechanistic studies reveal that TD-1's enhanced activity stems from its tighter binding affinity to EIF4G1, improved molecular stability, and superior cellular bioavailability. Notably, TD-1 also exhibits a unique ability to inhibit Repeat Associated Non-AUG (RAN) translation, a process implicated in the pathogenesis of several neurological disorders including Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). These findings position TD-1 as a promising oligonucleotide therapeutic capable of repressing both canonical and RAN translation.

#26 - Gut Commensal Metabolites Modulate *V. cholerae* Biofilm Growth

Katherine Kirby, Heidi Pauer, Alyssa Pold, Caetano Antunes

Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

Microbes living in the human gut engage in complex interactions, resulting in protection of their host against pathogens. One such pathogen, *Vibrio cholerae*, infects the intestine and uses motility and biofilm formation as important virulence factors. Our lab previously identified *Enterocloster citroniae* as a gut microbiota member that produces metabolites with a dampening effect on *V. cholerae* motility while increasing biofilm formation. Our goal is to isolate additional gut microbiota members that show biological activity against *V. cholerae*, particularly those that inhibit biofilm formation. After isolating gut microbes from fecal samples, we used ethyl acetate to extract their metabolites and performed a biofilm formation assay to demonstrate the effects of these gut commensal extracts on *V. cholerae* biofilm formation. 41 strains were tested; of these, 10 showed a significant increase in *V. cholerae* biofilm formation while 9 showed a significant decrease. Using 16S rRNA sequencing, we have identified strains of

Flavonifractor plautii and *Neglecta timonensis* as biofilm-inducing. Strains that did not modulate *V. cholerae* biofilm production have been identified as *Clostridium tyrobutyricum*, *Staphylococcus epidermidis*, *Cutibacterium acnes*, *Bacillus licheniformis*, and *Anaerotruncus colihominis.* Other strains are still in the process of being identified, including the biofilm-inhibiting strains. After this we will continue to test bioactive extracts for other effects on *V. cholerae* virulence and identification of specific bioactive small molecule(s).

#27 - Presenilin Loss-of-Function Mutations Do Not Lead to Synaptic Degeneration and Reduced Lifespan in *C. elegans*

Caitlin L. Libowitz[1], Brian D. Ackley[2], Michael S. Wolfe[3]

[1]Graduate Program in Neurosciences, Department of Pharmacology & Toxicology, University of Kansas, Lawrence, KS, USA; [2]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; [3]Department of Medicinal Chemistry, University of Kansas, Lawrence, KS, USA

Familial Alzheimer's Disease (FAD) is caused by autosomal dominant mutations in three genes: Amyloid Precursor Protein (APP), Presenilin 1, and Presenilin 2. FAD follows the same pathology and progression of sporadic cases of Alzheimer's Disease, but is early-onset. Presenilin 1 forms the catalytic component of the enzyme complex γ-secretase, which cleaves APP. Cleavage of APP leads to the release of extracellular amyloid-β which is found in the amyloid plaques in Alzheimer's patients. While plaques are strongly associated with disease, focus on eliminating or treating them has yielded little in effective treatments and therapeutics. Our lab has developed a novel amyloid-independent hypothesis to further explore mechanisms of disease. FAD mutations in Presenilin and APP have the potential to lead to stalling of the enzyme-substrate complex, disrupting proteolysis. It is this stalling that we hypothesize to lead to neurodegeneration. Utilizing *Caenorhabditis elegans* as a genetic animal model, we developed transgenic animals containing various FAD mutants and analyzed them for synaptic degeneration and reductions in lifespan. In most cases, FAD mutants led to age-dependent synaptic degeneration and reduced lifespan. Mutation of a catalytic aspartate in Presenilin did not show the phenotype, but did show a visible egg-laying defect similar to that seen in *sel-12* mutants, the endogenous *C. elegans* Presenilin. These data indicate that a simple loss of function of human Presenilin is not sufficient to cause the phenotype, but that human and *C. elegans* Presenilins may be acting in a similar mechanism.

#28 - Identification of Interneurons in Stress Resistance And Longevity

Mingyi Liu, Shelby Innes, Iryna Graham, Lizzie Vetter, Shijiao Huang

Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS, USA

Abstract: Organisms are exposed to various environmental stress throughout their life, including heat, hypoxia, oxidative and pathogenic stress, which trigger stress responses in cells. These stress responses play critical roles in survival and lifespan. According to

reported research, increased stress resistance is generally
associated with extended lifespan. Neural signaling has been reported to be involved in stress response and regulatory of longevity. Some sensory neurons were known to regulate stress response. Interneurons are essential components of the nervous system and they integrate signals from sensory neurons. But the roles of interneurons in stress response and longevity remain poorly understood. We aimed
to identify interneurons involved in regulating stress response and longevity. Based on that, we can get a better understanding of the neural mechanisms that regulate aging. We employed various stress assays with neuron-specific ablation strains of *C. elegans* to screen the interneurons involved in stress response and longevity regulation. We chose some interneurons as candidates based on known neural circuit and connectome analysis research. One of the findings was that PVQ ablation worm
showed increased resistance to heat stress, mitochondria stress and endoplasmic reticulum stress. Which indicates that PVQ neuron inhibit these stress response pathways. We studied the role of PVQ neurons in longevity pathways at the same time. We tested four longevity pathways including mitochondrial associated longevity pathway, hypoxia-related longevity pathway, mitochondria associated pathway and dietary restriction pathway but all of them still work without PVQ neurons. Which suggests that the PVQ neurons are not necessary in these four longevity pathways. We are planning to figure out the role of PVQ neurons in stress response and get more interneurons involved to reveal the circuit of stress response in future work.

Key words: stress response, interneuron, longevity pathway

#29 - Investigating the Effects of H-bonding in Mn^{III}-hydroxo Complexes Participating in Proton Coupled Electron Transfer Reactions

Markell J. Lomax [1] , Priya Singh [1], Adedamola Opalade [1], Brandon B Nguyen [1], Martin Srnec [2], Timothy A Jackson [1]

[1]Department of Chemistry, University of Kansas, Lawrence, KS, USA; [2]The Czech Academy of Sciences, Prague, Czechia, CZ

In the active site of the enzymes manganese superoxide dismutase and manganese lipoxygenase, the Mn-^{III}OH/Mn-^{II}OH$_2$ redox couple enables proton-coupled electron transfer (PCET) reactions. Amino acids hydrogen bond to the hydroxo ligand, but the functional importance of this interaction is unclear. Previously, we studied the role that hydrogen bonding plays in PCET reactions for two synthetic model complexes, $[Mn^{III}(OH)(PaPy_2N)]^+$ and $[Mn^{III}(OH)(PaPy_2Q)]^+$. In the $[Mn^{III}(OH)(PaPy_2N)]^+$ complex the hydroxo ligand hydrogen bonds with a naphthyridine while the $[Mn^{III}(OH)(PaPy_2Q)]^+$ lacks this hydrogen bond. We found that hydrogen bonding enhances the rate of reaction for PCET between Mn^{III}-hydroxo complexes and different substrates. In this study we explore the effect of hydrogen bonding on the basicity of the Mn^{III}-hydroxo complexes. Titrations of Mn^{III}-aqua complexes revealed that $[Mn^{III}(OH)(PaPy_2N)]^+$ is more basic than $[Mn^{III}(OH)(PaPy_2Q)]^+$, which leads to a larger driving force for PCET reactions. A thermochemical analysis showed that for less acidic substrates there is a linear correlation between the driving force and rate of

reaction, while more acidic substrates deviate from the trend. We propose that the deviation from the trends is caused by a change in mechanism from a concerted proton electron transfer (CPET) to a proton transfer (PT).

#30 - Design, Synthesis, and Screening of Head-to-Tail Cyclic Peptide Libraries Against MDM2

Hyma Mandapaka, **Channa Herath**, Kendah Ballout, Haifan Wu

Department of Chemistry and Biochemistry, Wichita State University, Wichita, KS, USA

Cyclic peptides constitute a promising class of molecules for modulating protein–protein interactions (PPIs) due to their conformational rigidity, structural diversity, and resistance to proteolytic degradation. Consequently, considerable efforts have been directed toward the discovery of bioactive cyclic peptides through peptide design and screening. A challenge inherent in cyclic peptide screening is the accurate sequencing, as tandem mass spectrometry, commonly employed for sequencing linear peptides, frequently produces false positives. Accordingly, various encoding strategies have been developed, including coding with linear peptides, mRNA, and other methods. In this study, we designed and synthesized large libraries of head-to-tail cyclic peptides and screened them against the N-terminal domain of MDM2, a critical negative regulator of p53. Our strategy enabled efficient generation, purification, and sequencing of cyclic peptides, leading to the identification of potential binders with high affinity to MDM2. This platform demonstrates the feasibility of ultra-diverse cyclic peptide libraries for targeting challenging PPIs and sets the stage for developing novel peptide-based therapeutics.

#31 - Strategies for Synthesis and Functionalization of 14-Membered Sulfamide Macrocycles

Dimuthu Vithanage, **Anna C. Marstall**, James B. Martinez, Alec S. Lininger, and Paul R. Hanson

Department of Chemistry, The University of Kansas, Lawrence, KS, USA

While macrocycles are generally well-known for their potential for biological activity, those containing sulfur are relatively underpopulated in the literature. Our interest in the development of novel sulfur-containing heterocycles leads us to report the development of a strategy for facile synthesis of 14-membered sulfamide-containing macrocycles. In this pot-efficient 3-component approach, amino and alcohol components possessing sp3-rich stereogenic centers, as well as olefins/acetylenes, are coupled with chlorosulfonyl isocyanate, followed by *N*-alkylation. Subsequent ring-closing metathesis and ring-closing enyne metathesis have been utilized for macrocyclization. The resulting 14-membered macrocyclic sulfamides possess three differentially-protected nitrogen atoms, allowing for selective deprotection and installation of functional groups. This synthetic strategy has potential for the generation of a diverse array of analogs, as each of the components used for coupling can be functionalized separately to produce a large variety of sp3-rich scaffolds.

#32 - Precision Cross-linking to Inhibit Tau Aggregation

Thomas McAdam, Emmanuel Ajiboye, Leo Hays, Haifan Wu

Department of Chemistry and Biochemistry, Wichita State University, Wichita, KS, USA

Alzheimer's disease and other tauopathies are characterized by the aggregation of tau protein. Previous studies have identified key residues in the repeat 2 (R2) and repeat 3 (R3) regions in tau as amyloidogenic motifs. Mutations and other factors that expose these motifs are known to promote tau aggregation. There are only two cysteine residues in 4R tau, one in R2 and the other in R3, which serve as sites for chemical cross-linking. Therefore, we hypothesized that cross-linking two cysteine residues can prevent the exposure of amyloidogenic motifs, thereby inhibiting tau aggregation. To test this hypothesis, we synthesized a thiol-reactive chemical cross-linker by attaching 6-maleimidohexanoic acid to the amino groups on the side chain and N-terminus of lysine. Successful cross-linking of tau was confirmed by mass spectrometry. SDS-PAGE analysis revealed that precision cross-linking of tau reduced the amount of heparin-induced tau aggregation. This study suggests that cross-linking cysteine residues in tau can modulate tau aggregation, which could be a new therapeutic strategy to treat tauopathies.

#33 - Flow Cytometry Core: A Chemical Biology of Infectious Disease COBRE Core Laboratory

Peter R. McDonald[1], Robin C. Orozco[1,2], and P. Scott Hefty[1,2]

[1]Flow Cytometry Core, Chemical Biology of Infectious Disease, University of Kansas, Lawrence, KS, USA; [2]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

The University of Kansas Flow Cytometry Core (FCC) provides access to flow cytometry and cell sorting instrumentation and expertise to researchers. Services and training are provided for flow cytometry: cell sorting and multi-parametric analysis of individual cells in solution, calculated from their fluorescent or light scattering characteristics. The FCC provides assistance in sample processing, data analysis, instrument training, software support, method and grant assistance, manuscript support, and consulting. The FCC is a 980 ft2 BSL-2 facility equipped with BD FACSymphony S6 and FACSAria Fusion cell sorters, a Cytek Aurora spectral flow cytometer, an Agilent NovoCyte Advanteon conventional flow cytometer, and other supplemental assay instrumentation (Bio-Rad QX600 ddPCR, C.T.L ImmunoSpot). The Cytek™ Aurora full-spectrum flow cytometry provides users with both tube-based and 96-well plate based spectral cytometry, with 5 lasers to allow analysis of 30+ colors. The BD FACS instruments allow measurement and sorting of up to 6 resolved populations of cells simultaneously, based on up to 50 parameters of detection using 18 simultaneous fluorochromes. The facility is equipped to handle BSL-2 samples and perform aseptic and single cell sorting into tubes or 96-well plates. The facility provides instrument training for users who desire to become self-operators of the facility instruments. The FCC will equip CBID researchers with tools directly applicable to infectious disease research, such as identifying and characterizing

infectious agents such as bacteria and parasites, quantification and sorting of cells infected with microbial pathogens, and assessing chemical probe efficacy against infectious agents. The FCC resources enable monitoring immune responses and activation status associated with infection, and measuring changes in cellular phenotypes (size, granularity, complexity, density, expression) in response to compound treatment. The FCC seeks to assist CBID collaborators in achieving their research goals.

Scientific Focus Area: Core Facility

Grant Support:

P20 GM113117/NIGMS NIH HHS/United States Chemical Biology of Infectious Disease

#34 - Metformin Suppresses Autophagy by Inhibiting Phagophore Tethering

Thuraya M. Mutawi[1], Udita Shah[2], Carlo Barnaba[1,3]

[1]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, USA; [2]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; [3]University of Kansas Cancer Center, Kansas City, KS, USA.

Autophagy is a conserved degradation pathway that enables cancer cells to survive metabolic stress. The AMP-activated protein kinase (AMPK) is a central regulator of energy balance, and its pharmacological activation by metformin — an FDA-approved antidiabetic drug — has led to growing interest in metformin as an anti-cancer therapy. However, the effect of AMPK activation on autophagy flux remains controversial. In this study, U2OS cells were treated with metformin and analyzed using western blotting and fluorescence microscopy. Western blots revealed that metformin, even at concentrations exceeding clinical levels (>5 μM), did not enhance p62 degradation. Live-cell imaging with Halo-tagged ATG13 and WIPI2 with GFP-p62 and GFP-LC3 reporters further demonstrated that metformin activation of AMPK causes a decrease in phagophore firing, which then results in a significant decrease in autophagosome maturation. Finally, single-molecule live-cell imaging on ATG13 suggests that metformin, similar to glucose starvation, blocks autophagy by dampening phagophore tethering to the source membrane. These findings suggest that metformin, through AMPK activation, may inhibit rather than promote autophagy, highlighting its complex role in cancer metabolism and its therapeutic implications.

#35 - The ABC transporter EF2223-EF2221 of *Enterococcus faecalis* Imports High Mannose Glycans, and is Dependent on a Three-component Signal Transduction System

Abdulrahman M. Naeem[1], Tolulope I. Ade[1], Zakria H. Abdullahi[1], Rupa Addanki[2], Mark P. Farrell[2], Ana Flores-Mireles[3], and Lynn E. Hancock[1]

[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; [2]Department of Medicinal Chemistry, University of Kansas, Lawrence, KS, USA; [3]Department of Biological Sciences, Notre Dame University, South Bend, IN, USA

In the absence of preferred carbon sources, the opportunistic pathogen, *Enterococcus faecalis*, utilizes a diverse array of carbon substrates for metabolism including host glycans. Through transcriptomic studies, we identified a 6 gene operon comprised of an ABC transporter and signal transduction system that was abundantly expressed in the presence of high mannose N-linked glycans. Using luciferase reporter assays, we show that operon expression is dependent on the signal transduction system, in particular the response regulator YesN, and requires endoglycosidase activity to liberate the glycans from glycoproteins. We hypothesized that YesN would contribute to physiological adaptations at sites of infection and tested the parental and *yesN* mutant in a catheter associated urinary tract infection model in mice. The *yesN* mutant was attenuated in dissemination to distal sites from the initial inoculation in the bladder, suggesting that this glycan sensing and import system are important to *E. faecalis* as a pathogen. We are presently testing the ability of the ABC transporter to recognize distinct host glycans for nutrient uptake. We have purified the solute binding protein, EF2221, and will be testing several distinct forms of high mannose glycans for binding affinities using microscale thermophoresis. We are also interested in the signal transduction pathway connecting the histidine kinase, YesM, for its ability to phosphorylate YesN to initiate signal transduction. We have purified the cytoplasmic domain of YesM and the YesN protein and will be conducting phosphotransfer assays along with gel mobility shift assays to establish their function in signal transduction.

#36 - Metabolites Produced by Bacterial Skin Microbiome Members Disrupt *Staphylococcus aureus* Biofilm For mation

Aidan O'Hara, Rami Hanna, Breanna Wuerzberger, Rosana B. R. Ferreira

Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

The skin microbiome consists of microorganisms, including bacteria, viruses, fungi, and other eukaryotic microorganisms. Recent research has revealed that certain members of the skin microbiome can reduce the pathogenicity of bacterial skin pathogens, such as *Staphylococcus aureus*. One major virulence factor of *S. aureus* is its ability to produce biofilms, which allow the bacteria to secure an ecological niche in harsh environments. Additionally, biofilms confer enhanced resistance towards antimicrobials and the host's immune response to biofilm-producing bacteria, facilitating hard-to-treat infections. Novel strategies must be developed that

either decrease the biofilm production of *S. aureus* or its pathogenicity to improve *S. aureus* infection outcomes. The goal of this research was to identify specific bacterial skin microbiome members that produce metabolites that affect *S. aureus*' biofilm production. Species identification was performed using Matrix-Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry (MALDI-ToF MS). Metabolites were sourced from the cell-free conditioned media (CFCM) of bacterial skin isolates grown for 24 hours with aeration. These CFCMs were then assayed for their activity against *S. aureus* biofilm production or growth rate with a biofilm assay or growth curve assay, respectively. We have found that almost all *Staphylococcus epidermidis* strains tested instilled a significant reduction in *S. aureus'* biofilm production independent of growth. *Bacillus* species' CFCM also showed an ability to decrease *S. aureus'* biofilm production. However, some *Bacillus* CFCMs reduced *S. aureus'* growth rate. Species of *Corynebacteria* have had variable activity against *S. aureus'* biofilm production, where some strains increase and others decrease production. Both phenotypes occur independent of changes in *S. aureus'* growth rate. The research presented herein showcases that production of metabolites by bacterial skin microbiome members that affect *S. aureus*' biofilm production in a growth-independent manner is common, emphasizing the bacterial skin microbiome metabolome as an attractive reservoir for biomolecules that decrease the production of a key *S. aureus* virulence factor.

#37 - The Synthetic Chemical Biology Core (SCB): A Resource for Research in Chemical Biology

Chamani Perera

Synthetic Chemical Biology Core Laboratory, University of Kansas, Lawrence, KS, USA

The Synthetic Chemical Biology Core strives to provide comprehensive synthetic chemistry capabilities to investigators under one roof. The synthetic expertise of the core includes, but is not limited to, novel and commercially unavailable small molecules, fluorescent molecules and custom peptides. The core assists in identifying hits for medicinal chemistry optimization in infectious disease targets and provides synthesis capabilities for structure activity studies of said hits. The core staff will work with investigators to design and synthesis novel molecular probes to facilitate their research. SCB core provides purification, analysis and quality control of compounds via LC/MS. The SCB core also offers MALDI-TOF analysis of biomolecules.

#38 - Modeling ApoE/TREM2 Complex Using Cross-linking Mass Spectrometry and Protein-Protein Docking

Doniesha L. Perera, Emmanuel Ajiboye, Haifan Wu

Department of Chemistry and Biochemistry, Wichita State University, Wichita, KS, USA

Alzheimer's disease (AD) is a devastating neurodegenerative disease with few disease-modifying therapies. Human genetics studies have identified two major genetic risk

factors for late-onset AD—apolipoprotein E (*APOE*) and triggering receptor expressed on myeloid cells 2 (*TREM2*). ApoE binds to the low-density lipoprotein receptor (LDLR) to facilitate the uptake of ApoE-lipoprotein particles, while TREM2 is a cell surface receptor expressed on microglia in the brain. The activation of TREM2 is essential for microglial survival, proliferation, and phagocytosis in order to carry out their protective functions against AD pathology. Recently, several studies have shown the activation of TREM2 signaling through the direct interaction between TREM2 and ApoE. In addition to the important role of ApoE/TREM2 interaction in AD pathogenesis, this interaction has been shown to induce immunosuppression of neutrophils within the tumor microenvironment. Therefore, a detailed understanding of this interaction could lead to novel therapeutic strategies targeting ApoE and TREM2. Although biophysical studies have been carried out to characterize this interaction, there is still no detailed structural model. Here we carried out chemical cross-linking of the ApoE/TREM2 complex followed by bottom-up mass spectrometry analysis to identify inter-protein cross-links, which were used as distance restraints to guide protein-protein docking using Haddock. We obtained the first structure model of the ApoE/TREM2 complex. We believe this model will facilitate future research of designing probe molecules to better understand the physiological functions of the ApoE/TREM2 interaction.

#39 - Identification of a Series of Pyrrolo-pyrimidine based SARS-CoV-2 Mac1 Inhibitors that Repress Coronavirus Replication

Jessica J. Pfannenstiel[1], Michelle D. Woolridge [2] Men Thi Hoai Duong[3], Daniel Cluff[1], Lavania M. Sherrill[4], Iain Colquhoun[4], Gabrielle Cadoux[4], Devyn, Thorne[4], Johan Pääkkönen[3], Nathan Schemmel[1], Joseph O'Connor[1], Pradtahna Saenjamsai[1], Mei Feng[5], Michael J. Hageman [5,6], Andrew Johannesen[7], David K. Johnson[7], Anu Roy[8], Lari Lehtiö, Dana V. Ferraris[4], Anthony R. Fehr[1]

[1]Department of Molecular Biosciences, University of Kansas, Lawrence, Kansas, USA; [2]Faculty of Biochemistry and Molecular Medicine & Biocenter Oulu, University of Oulu, Oulu, Finland; [3]McDaniel College Department of Chemistry, 2 College Hill, McDaniel College, Westminster, Maryland 21157, USA; [4]Biopharmaceutical Innovation & Optimization Center, University of Kansas, Lawrence, Kansas, USA; [5]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas, USA; [6]Molecular Graphics and Modeling Laboratory and the Computational Chemical Biology Core, University of Kansas, Lawrence, Kansas, USA; [7]Infectious Disease Assay Development Laboratory/HTS, University of Kansas, Lawrence, Kansas, USA

Coronaviruses (CoVs) can emerge from zoonotic sources and cause severe diseases in humans and animals. All CoVs encode for a macrodomain (Mac1) that binds and removes ADP-ribose from target proteins. SARS-CoV-2 Mac1 promotes virus replication in the presence of interferon (IFN), though the mechanisms by which it promotes replication remain unknown. Inhibitors of Mac1 could help elucidate these mechanisms and serve as potential therapeutic agents against CoV-induced diseases. We previously identified compound **4a**, a pyrrolo-pyrimidine that inhibited Mac1 activity *in vitro* at low micromolar concentrations. Here, we determined the binding mode of **4a** by X-ray

crystallography, further characterizing its interaction with Mac1. However, **4a** did not reduce CoV replication in cells, which we hypothesized was due to poor cellular permeability. To address this, we developed several hydrophobic derivatives of **4a** and identified four compounds that inhibited both Mac1 in vitro and murine hepatitis virus (MHV) replication, including compounds **5a** and **5c.** Notably, **5c** also inhibited SARS-CoV-2 replication, but only in the presence of IFN-γ, mirroring the phenotype observed with a Mac1 deletion virus.

To confirm specificity, we serially passaged MHV in the presence of **5c** to identify drug-resistant mutations. Following passaging with **5c**, we identified a double base-pair mutation (G1386E). A recombinant virus engineered with this mutation exhibited enhanced replication compared to wild-type virus when treated with 5c, confirming the development of drug resistance. Additionally, protein modeling suggests that this mutation promotes preferential binding to ADP-ribose over 5c, thereby reducing the effectiveness of the inhibitor. These findings provide proof of principle for the development of Mac1-targeted therapeutics.

#40 - *PTPN22* Impacts the B Cell Landscape During Chronic Virus Infection

Macie A. Proctor-Roser, Tammy R. Cockerham, and Robin C. Orozco

Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

B cell dysfunction is associated with persistent virus infections. Improving B cell functions, like earlier antibody production or enhanced APC ability, could improve virus clearance. Mice lacking the immune regulatory gene *Ptpn22* (PEP-null) clear persistent virus strain LCMV-cl13, whereas wildtype mice (PEP-WT) do not. During autoimmunity, *Ptpn22* regulates BCR signaling, affecting activation and differentiation. However, how *Ptpn22* impacts B cell dynamics during virus infection remains poorly understood**. Based on the findings of *Ptpn22* in B cells during autoimmunity, we hypothesize that PEP-null mice will have an altered B cell landscape during virus infection, possibly leading to clearance.** First, we profiled B cell subsets in spleen, lymph nodes, and bone marrow from PEP-WT and PEP-null mice at naïve and various days post LCMV-cl13 infection. In naïve splenocytes, PEP-null mice had higher frequency of Follicular and Plasmablasts than PEP-WT. Post infection, PEP-null had less Follicular, but more Plasmablasts compared to PEP-WT in spleen. The inverse was seen in the lymph nodes. In line with this, PEP-null mice had increased serum levels of anti-LCMV IgG2A at 9DPI. Additionally, we determined PEP-null B cells had increased MHC-II but decreased PD-L1 expression in both spleen and lymph nodes. Ongoing studies detail T cell-B cell interactions that may be regulated by *Ptpn22*. Results strongly indicate *Ptpn22* is a key regulator of B cell dynamics during persistent virus infection.

#41 - Ferric and Manganese Hydroxide Complexes as Bioinspired Models of Lipoxygenase

Anagha Puthiyadath and Timothy. A. Jackson

Department of Chemistry, University of Kansas, Lawrence, KS, USA

In biology and synthetic chemistry, transition metal oxidation processes often involve coupled transfer of a proton and an electron. In many of these cases, metal-oxygen species (M=O or M-OH) initiate C-H or O-H bond oxidation by concerted proton-electron transfer (CPET) reactions that involve electron transfer to the metal and proton transfer to the oxygen ligand. Enzymes like lipoxygenase (LOXs) use M-OH to oxidize fatty acids. Traditionally associated with iron, LOXs were later found to utilize manganese in the pathogenic fungi *Gaeumannomyces graminis* and *Magnaporthe oryzae* that target wheat and rice. While Fe-LOXs are widespread in plants and animals, Mn-LOXs appears only in certain fungi. The examination of Fe^{III}-hydroxo and Mn^{III}-hydroxo model complexes is significant in understanding the initial substrate oxidation step in LOXs. The scarcity of Mn^{III}-hydroxo and Fe^{III}-hydroxo complexes with the same coordination sphere poses a challenge in understanding the steric and electronic factors that govern the thermodynamic and kinetic properties of these complexes. This work discusses the generation and examination of the properties of a Fe^{III}-hydroxo complex, $[Fe^{III}(OH)(dpaq)]^{+}$and compare it to already characterized Mn^{III} hydroxo analogue. Electrochemical techniques are used to calculate the Bond Dissociation Free Energy (BDFE) of Fe^{III}-hydroxo complex and compare to the Mn^{III}-hydroxo analogue. When combined with kinetic studies of CPET reactions by both Mn^{III}-hydroxo and Fe^{III}-hydroxo complexes, this approach yields a comparison of the thermodynamic properties and reactivity of metal (III)-hydroxo complexes at parity of coordination sphere.

#42 - Unpacking Individual Amino Acid Contributions of the RGD-motif Mediating the Function of the $\alpha V\beta_3$ Integrin In Silico

Dilanka R. Ranaweera[1], John Hoopes[1], Justin Carbone[2], and Krzysztof Kuczera[1,2]

[1]Department of Chemistry, University of Kansas, Lawrence, KS, USA; [2]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

Arginine-glycine-aspartate (RGD) is a highly conserved amino acid motif present in various Extracellular Matrix (ECM) proteins, including fibronectin, vitronectin, and osteopontin. This motif plays a significant role in regulating processes such as cancer progression, metastasis, and angiogenesis, acting especially through its interaction with RGD-binding integrins. The αVβ3 integrin, a member of the RGD-binding integrin subfamily, binds to the RGD motif via coordination of a divalent metal ion at the Metal Ion-Dependent Adhesion Site (MIDAS), hydrogen bonding, and van der Waals interactions at the interface of the α and β subunits. To better understand the role of individual residues in the RGD motif, we explored interactions with αVβ3 integrin for all possible three-amino acid sequence variations. 8,000 variants of the cyclic peptide

XXXFV, with X denoting the variable sites, were generated and docked to the integrin with AutoDock CRANKPEP (ADCP). Interestingly, a large number of tested sequences exhibited calculated binding affinities to αVβ3 integrin higher than that for the parent RGDFV. After experimental verification and testing of the pharmacological properties of the discovered peptides, these systems may be the basis of new and improved integrin-targeted therapies.

#43 - Infectious Disease Assay Development Core: High Throughput Screening Laboratory at the University of Kansas

Anuradha Roy, PhD

IDAD-HTS Laboratory, University of Kansas, Lawrence, KS, USA

The overall goal of the IDAD Core is to provide expertise, facilities, services, and training in the area of HTS assay design, development, validation, small and large-scale screening for whole cell based or biochemical infectious disease targets. The IDAD core is an extension of the University of Kansas High Throughput Screening Laboratory which is a fee-for-service, state-of-the-art facility dedicated to providing academia, not-for-profit institutions, biotech, and pharmaceutical industries with exceptional assay development, high throughput screening and data mining services at economical rates. The staff has experience in executing cell-based, biochemical, siRNA as well as high content screening campaigns against a plethora of target classes. The laboratories are equipped with cutting-edge liquid handling and signal detection instrumentation for increasing throughput and precision of screening campaigns. Clients have the option of using our collection of 395,000 compounds and/or a client's own chemical library. KU-IDAD/HTS lab further leverages the strengths of the medicinal chemistry/ computational modeling cores under CoBRE Chemical Biology of Infectious diseases (CBID) program to support your tool/lead discovery research.

#44 - Interactions Between the *Pseudomonas aeruginosa* Las Quorum-Sensing System and Stringent Response Reveal Distinct Mechanisms of Resistance to Ceftazidime and Piperacillin

Vanessa M. Schmidt, Brielle M. Mckee, Josephine R. Chandler

Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA

In *Pseudomonas aeruginosa,* the acyl-homoserine lactone (AHL)-dependent *las* quorum-sensing system enhances resistance to multiple antibiotics, including the clinically important β-lactam ceftazidime. β-lactams susceptibility is also influenced by the stress-inducible stringent response system, which increases detoxification of reactive oxygen species (ROS) in non-growing cells to maintain membrane integrity and mitigate antibiotic internalization. Here, we examine the interaction of LasR with stringent response (SR) in shaping resistance to the β-lactam antibiotics ceftazidime and piperacillin. Our results showed that disrupting LasR decreases resistance to ceftazidime but not piperacillin. LasR also increases ROS levels and uptake of ethidium bromide, a

proxy for measuring membrane permeability, similar to that of the SR system. We could restore ceftazidime resistance and membrane resistance in the Δ*lasR* mutant by chemically or enzymatically detoxifying ROS. These results suggest LasR increases ceftazidime resistance through a shared pathway of ROS detoxification and protecting membrane integrity. In support of this idea, concurrently disrupting both the LasR and SR systems resulted in a non-additive effect on ceftazidime resistance that was similar to that of disrupting the individual systems. In contrast, we observed a different pattern for piperacillin. Individually disrupting LasR or SR had little to no effect on resistance, while disrupting both systems caused >10-fold decrease in resistance, revealing synergistic effects of these two systems that did not correspond to membrane permeability changes. These results indicate LasR and SR confer piperacillin through synergistic but different mechanisms potentially unrelated to membrane permeability. Together, our work reveals new mechanistic insights into how LasR and stringent response contribute to β-lactam antibiotic resistance that may be relevant to understanding the variables that influence *P. aeruginosa* evolution in complex environments, including infections.

#45 - Elucidating Immunomodulatory Mechanism of Bifunctional Peptide Inhibitors using Mouse Model of EAE

Andrea L. Villela[1,2], Su Jeong Song[1], Teruna Siahaan[1,2], Hyunjoon Kim[1,2]
[1]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, USA; [2]Bioengineering Program, University of Kansas, Lawrence, KS, USA

Purpose: According to the National Institutes of Health, up to 23.5 million people in the United States suffer from autoimmune diseases and their prevalence is rising.[1] Current treatments rely on immunosuppressants which halt immune cells from recognizing and attacking autoantigens. However, this broad suppression of the patients' immune system can increase their risk of infection and make them susceptible to other diseases. Further research is needed to find and develop antigen-specific immunotherapies that will regulate the autoreactive portion of the immune system while continuing specificity to fight foreign pathogens. This study aims to evaluate and understand the effects of different routes of administration for bifunctional peptide inhibitors (BPI), previously known to suppress experimental autoimmune encephalomyelitis (EAE) in an antigen-specific manner. BPIs are hypothesized to bind to both the MHC-II and to the costimulatory receptor ICAM-1 on antigen presenting cells (APC) and therefore block the immunological synapse which then alters the phenotype of naïve T cells from proinflammatory to a regulatory response.
Methods: Mouse bone-marrow derived dendritic cells (BMDCs) and splenocytes were harvested from C57BL/6 mice. In in vitro BMDC activation assays as well as naïve splenocytes assay, cells were plated and treated with myelin oligodendrocyte glycoprotein (MOG38–50) and its bifunctional peptide inhibitor (MOG-BPI) at a concentration of 10 μM. After 48 h incubation, cells were stained for flow cytometry analysis. Same parameters were used on RAW 264.7 cells to collect supernatant for lactate dehydrogenase (LDH) cytotoxicity assay. To investigate the in vivo efficacy of BPIs in different routes of administration, we established mouse multiple sclerosis model, which auto-reactive T cells attack the myelin sheath. On days 4 and 7 mice were injected with MOG-BPI either through intravenous injection (i.v.), subcutaneous injection (s.c.),

or intraperitoneal injection (i.p.) (n=5). The control groups with and without the induction of EAE were administered PBS via i.v. injection.

Results: When evaluating the cytotoxicity of the BPIs when using a 10 μM concentrations on RAW 264.7 cells there was little to no cytotoxicity found. When evaluating these peptides in vivo we observed an increase in Foxp3+ for all routes of administration but no difference amongst the groups. There was a difference among IL-10 an anti-inflammatory cytokine expression within the cells with the i.v. treated group having higher expression than the control or i.p. treated group. Additionally observed there was a significant decrease in expression for pro-inflammatory cytokine IL-17a among all groups but no significant difference among the different routes of administration. When observing the microglia extracted from brain tissue, we saw a decrease in expression in the i.v. and s.c. treated groups for costimulatory receptor CD86 known to be a pro-inflammatory marker. Additionally, we saw a decrease in pro-inflammatory cytokine TNF-α expression when administered through i.v. and s.c. although not significant.

Conclusions: In this study the route of administration affecting the function of the BPI was evaluated. Future studies can be done to improve the delivery of the peptides through nanoparticle formulation for local or systemic delivery. Pharmacokinetic studies to evaluate the clearance of the drug based on the route of administration. These improvements can help sustain the peptides for longer periods, therefore leading to long term therapeutic and reducing any relapses in EAE while the immune system keeps the specificity to fight other infections.

References:

1. NIH Autoimmune Diseases Coordinating Committee: Autoimmune Diseases Research Plan, March 2005, https://www.niaid.nih.gov/sites/default/files/adccfinal.pdf
2. Mahadik, R. Kiptoo, P. Tolbert, T. Siahaan, T. (2022) Immune modulation by antigenic peptides and Antigenic peptide conjugates for treatment of Multiple Sclerosis. *Med Res Arch.* doi:10.18103/mra.v10i5.2804

Acknowledgements: This work was supported in part by a grant from CoBRE-Chemical Biology of Infectious Disease (NIH award number P20GM113117) (H.K). TJS received Funding from National Institutes of Health R01-AG08227.

#46 - Nanoparticle-stabilized Pickering Emulsion for Oral Vaccine Delivery

Jin Xie, Grahmm A. Funk, Su Jeong Song, Xiaodi Li, Hyunjoon Kim

Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, USA

Introduction: The major challenge in oral vaccine delivery is overcoming gastric acid degradation while ensuring robust immune activation (1). Pickering emulsions, stabilized by solid particles, enhance stability and antigen transport while protecting against gastric degradation (2). Nanoparticles encapsulating the immunostimulant R848 (R848-NP) further improve efficacy by acting as stabilizers and adjuvants (3). Therefore, we designed an R848-NP-stabilized Pickering emulsion (R848-NP@PE-OVA), aiming to enhance antigen stability, uptake, and mucosal immunity.

Methods： R848-NPs were synthesized using the nanoprecipitation method, and R848-NP@PE-OVA was prepared via sonication. The characterization of R848-NP@PE-OVA was further assessed, including particle size, zeta potential, stability over a 7-day storage period, and stability in Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF). The uptake of R848-NP@PE-OVA by bone marrow-derived dendritic cells (BMDCs) was quantified via flow cytometry by measuring fluorescence in CD11c-positive cells. BMDC activation and cytotoxicity were evaluated through co-stimulatory molecule (CD40, CD86, CD80) expression and the lactate dehydrogenase (LDH) assay, respectively. The in vivo immune responses in mice were assessed by measuring serum IgG and fecal IgA levels using ELISA.

Results: R848-PLGA-NP@PE-OVA was successfully formulated with a uniform particle size (245 nm), a stable Zeta potential (-40 mV), and high drug loading (~80%), maintaining stability for seven days as well as in SGF and SIF. BMDC uptake analysis revealed an approximately threefold increase in OVA fluorescence within $CD11c^+$ BMDCs treated with R848-PLGA-NP@PE-OVA compared to free OVA. Cytotoxicity analysis demonstrated that BMDCs exposed to R848-PLGA-NP@PE-OVA (OVA: 2 μM–1 nM; R848: 28 μM–200 nM) maintained nearly 100% cell viability. BMDC activation analysis demonstrated that R848-PLGA-NP@PE-OVA significantly upregulated CD80 (15.3% to 17.9%, $P < 0.005$) and CD86 expression (27.9% to 39.9%, $P < 0.0001$) compared to free OVA+R848. In vivo immune response evaluation showed that the R848-PLGA-NP@PE-OVA group exhibited IgG levels approximately 3.9-fold higher than the PBS group and 2.5-fold higher than the free R848+OVA group. Similarly, IgA levels in the R848-PLGA-NP@PE-OVA group were significantly elevated compared to the PBS group ($P < 0.01$) and the free R848+OVA group ($P < 0.05$).

Conclusion: This study demonstrates that R848-PLGA-NP@PE-OVA enhances antigen uptake, dendritic cell activation, and immune responses, making it a promising vaccine adjuvant. It significantly improves BMDC maturation, antigen presentation, and systemic and mucosal immunity (IgG, IgA). These findings support PLGA-based Pickering emulsions as an innovative platform for mucosal vaccination.

Acknowledgements: This work was supported by National Institutes of Health (P20GM113117, CoBRE-Chemical Biology of Infectious Diseases)

References: (1) Ramirez, J. E. V., et al. Advanced drug delivery reviews, 2017: 116-131. (2) Du, Y., et al. Biomaterials, 2022: 121313. (3) Toy, Randall, et al. Journal of Controlled Release. 2021: 866-877.

#47 - Spinal Cord Microglia Exhibit Impaired Responses to Myelin Damage

Matthew C. Zupan and Meredith D. Hartley

Department of Chemistry, University of Kansas, Lawrence, KS, USA

Multiple sclerosis (MS) is the most common non-traumatic neurodegenerative disease affecting young adults. The onset of the disease is caused by a dysregulated immune response that promotes central nervous system (CNS) damage and myelin damage, or demyelination. Microglia, the resident immune cells of the CNS, have increasingly been implicated in orchestrating several aspects of myelin repair. They phagocytose myelin debris that builds up during demyelination, which allows for the subsequent recruitment of oligodendrocyte progenitor cells to the lesion site to initiate myelin repair. Microglia have also been shown to promote remyelination by secreting regenerative factors, modulating the extracellular matrix, and metabolizing lipids that can be recycled into new myelin. Some recent findings in our lab have shown that the brain and the spinal cord react very differently to myelin damage in our mouse model of demyelination. The brain shows a clear period of demyelination, followed by a clear phase of remyelination. On the other hand, the spinal cord only shows a phase of worsening demyelination with no signs of remyelination. It is surprising that these two tissues react so differently to myelin damage, and a better understanding of why the spinal cord struggles to remyelinate could enable us to better treat multiple sclerosis. The goal of this project is therefore to look into the difference between the brain and the spinal cord after myelin damage at the microglia level, since microglia are such a key player in orchestrating myelin debris clearance and initiating myelin repair. Through flow cytometry analysis, we have determined that brain and spinal cord microglia show different expressions of key cell surface proteins that are highly implicated in myelin damage and repair mechanisms. We have also observed differences in both microglia activity and protein expression in the brain compared to the small cord through immunofluorescence analysis of tissue slices. Furthermore, microglia morphology, which is directly connected to microglia health and function, changes drastically between the brain and spinal cord during myelin damage and repair. By further investigating these differences between the brain and the spinal cord, we hope to eventually elucidate specific mechanisms that cause these differences. This would help us not only have a more encompassing view of how to treat demyelinating diseases but unraveling these mechanisms that inhibit the spinal cord from remyelinating could additionally lead to currently unknown avenues for treating demyelinating diseases.