KU THE UNIVERSITY OF KANSAS

CHEMICAL BIOLOGY SYMPOSIUM 2024

OCT 25-26

3rd Annual Chemical Biology Symposium

Friday, October 25th – Jayhawk Welcome Center

4:00 – 4:45 Welcome session – Glorious to View Room

Check-in, Drinks, & Light Refreshments

General Greeting – Symposium Organizers

4:45 – 6:00 Keynote Presentation – Berkley Presentation Room

Matthew Russolillo, Session Chair

Dr. Matthew Bogyo – Professor of Pathology and of Microbiology and Immunology and, by courtesy, of Chemical and Systems Biology; Stanford University

"*Chemical Probes for Imaging Cancer and Infectious Diseases*"

6:00 – 7:00 Dinner (Taco Bar Buffet)

7:00 – 8:00 Lightning Talks! – Graduate Trainees

Eric Ebert, Session Chair

James Martinez, KU Department of Chemistry, Hanson Lab

"*Efforts Toward the Synthesis of α-Glucosidase Inhibitor Daphnepapytone A*"

Andrew Daufel, KU Department of Molecular Biosciences, Slusky Lab

"Characterization of TolC Colicin Interactions"

Markell Lomax, KU Department of Chemistry, Jackson Lab

"Exploration and Analysis of the Effects of Hydrogen Bonding on the Basicity of Mn^{III}-hydroxo Complexes in Proton-Coupled Electron Transfer (PCET) Reactions"

Erick McCloskey, KU Department of Molecular Biosciences, Davido Lab

"*HSV-1 ICP0 Dimer Mutants Impair Protein Functions and Viral Replication*"

Ashten Gentry, KU Department of Chemistry, Whelan Lab

"Addressing Racial Disparities in CA125 Detection Rates Using a "Multi-omics" Approach"

Saturday, October 26th – School of Pharmacy (SOP)

8:30 – 9:15 Morning Social session – SOP Atrium

Check-in, Light Refreshments

9:15 – 10:45 Session I (15-minute presentations followed by ~3 min questions) 2020 SOP

Alec Bevis, Session Chair

Welcome and brief opening comments – Symposium Organizers

Reed Stubbendieck – Assistant Professor, OSU Department of Microbiology and Molecular Genetics

"*Bacterial Competition in the Aerodigestive Tract: Antibiotics, Probiotics, and Beyond* "

Michael Johnson – Associate Professor, KU Department of Chemistry

"Sub-second Electrochemical Measurements of Oxytocin in Live Zebrafish Brains: Implications for Nociception"

Ming Zhao – Assistant Professor, KU Department of Pharmaceutical Chemistry

"*Harnessing the power of polymer-protein assemblies for multimodal cancer therapy*"

Rosana Ferreira – Assistant Professor, KU Department of Molecular Biosciences

"*Diffusible molecules produced by skin commensals and their role in colonization resistance*"

10:45 – 11:00 AM Break

11:00 – 12:00 PM Special Speaker – 2020 SOP

Alec Bevis, Session Chair

Tyrrell Conway, Regents Professor, OSU Department of Microbiology and Molecular Genetics

"*Nitrogen assimilation by E. coli in the mouse intestine*"

12:00 – 1:00 Lunch (Boxed Lunches Available), Mortar & Pestle, SOP Basement

1:00 – 2:00 Session II (15-minute presentations followed by ~3 min questions), 2020 SOP

Markell Lomax, Session Chair

Bao Vu – Assistant Professor, OUHSC Department of Microbiology and Immunology

"*Azole Drug Resistance in Nakaseomyces glabratus*"

Michael Wang – Professor, KU Department of Pharmaceutical Chemistry

"*CYP5122A1 encodes an essential sterol C4-methyl oxidase in Leishmania donovani and determines the antileishmanial activity of antifungal azoles*"

Luke Erber – Assistant Professor, KU Department of Medicinal Chemistry

"*Profiling DNA-Protein Crosslinks*"

2:00 – 2:15 Break

2:15 – 3:15 Session III (15-minute presentations followed by ~3 min questions), 2020 SOP

Markell Lomax, Session Chair

Hongbing Yu – Assistant Professor, KUMC Department of Microbiology, Molecular Genetics and Immunology

"*Neuro-immune-microbe interactions in the gut"*

Chamani Perera – Director, KU Synthetic Chemical Biology Core

"The Synthetic Chemical Biology Core (SCB): A Resource for Research in Chemical Biology"

Zarko Boskovic – Assistant Professor, KU Department of Medicinal Chemistry

"*How to discover bioactive small molecules (when all you have is synthetic*

chemistry and cell culture)?"

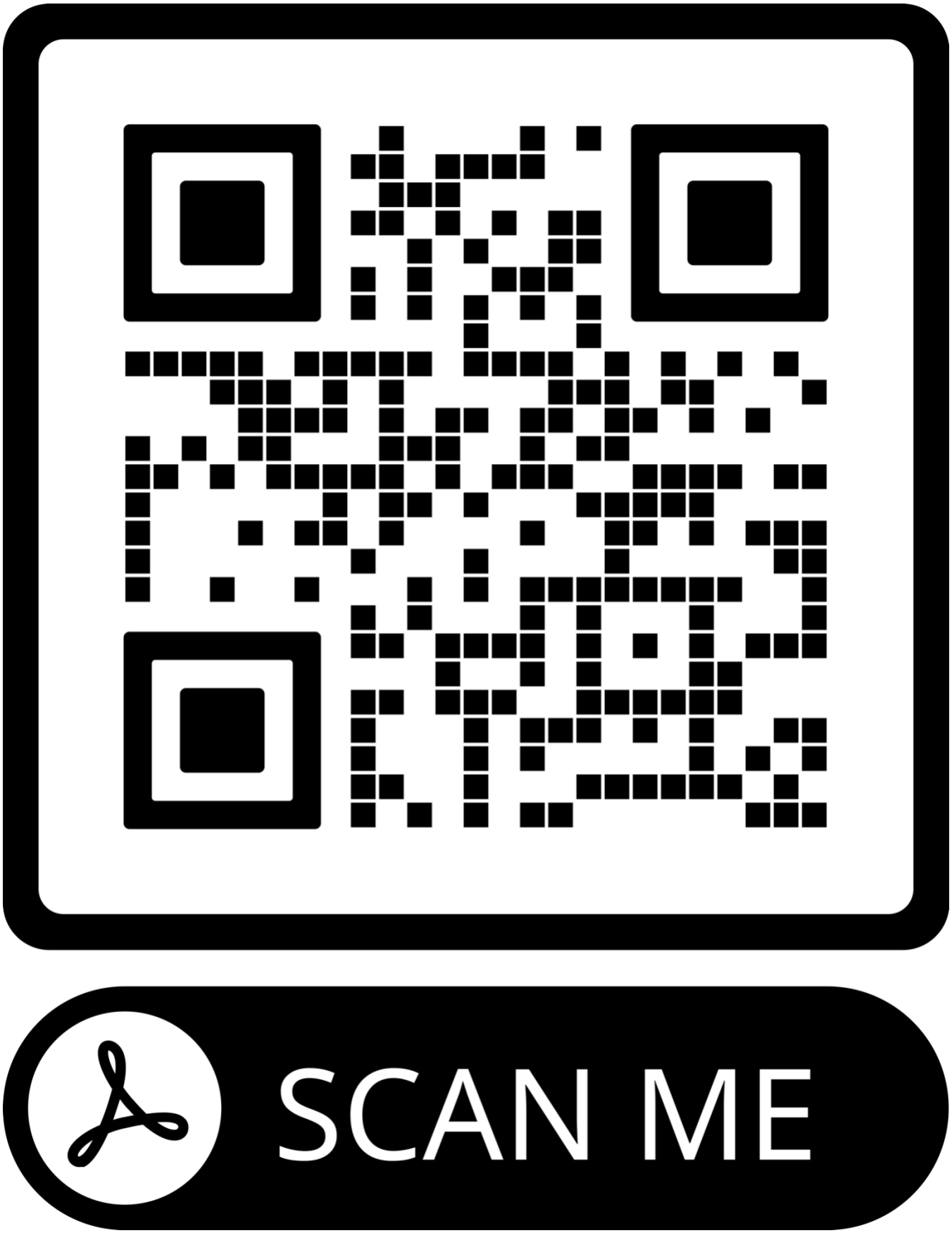
3:30 – 5:00 PM Poster Session (refreshments), SOP Atrium

3:30 – 4:15 Poster Session I (odd numbered posters)

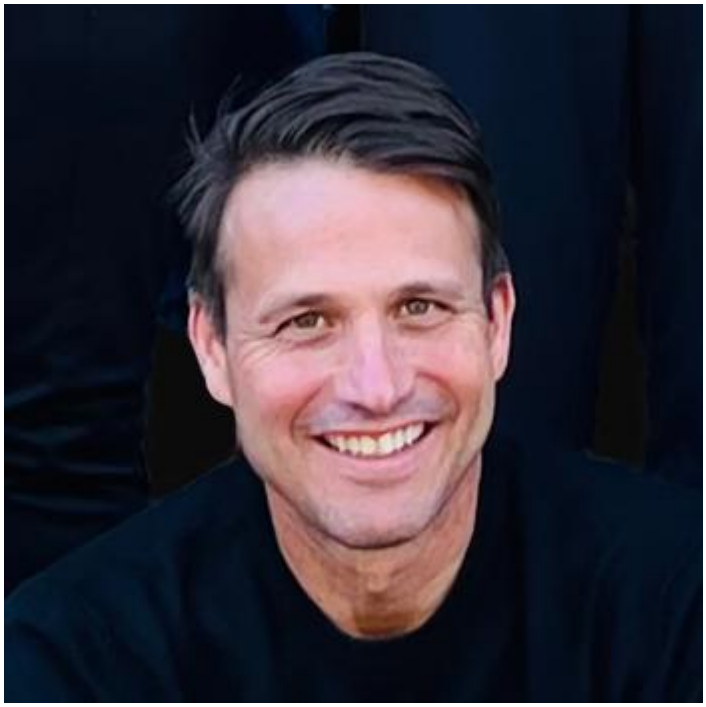
4:15 – 5:00 Poster Session II (even numbered posters)

5:00 – 5:20 Poster and Flash Talk Award Ceremony, 2020 SOP

5:20 Conclude


SCAN ME

Keynote Lecture – Friday, October 25th



Dr. Matthew Bogyo

Professor of Pathology and of Microbiology and Immunology and by courtesy of Chemical and Systems Biology

Department of Pathology

Stanford University School of Medicine

Stanford, CA, 94305-5324

Chemical Probes for Imaging Cancer and Infectious Diseases

Hydrolases are enzymes (i.e. proteases, esterases, lipases) that often play pathogenic roles in many common human diseases such as cancer, asthma, arthritis, atherosclerosis and infection by pathogens. Therefore, tools that allow dynamic monitoring of their activity can be used as diagnostic agents, as imaging contrast agents and for the identification of novel enzymes as drug leads. In this presentation, I will describe our efforts to design and build small molecule probes that can be used to identify, inhibit and image various hydrolase targets in models of cancer and infectious disease. This will include recent advances in protease activated fluorescent probes for real-time visualization of tumors during surgery as well our efforts to identify several new classes of serine hydrolases in pathogenic and commensal bacteria. We believe many of these enzymes will represent valuable imaging and therapy targets that can be used to visualize and disrupt various aspects of colonization and community formation inside a host.

Lightning Talks – Friday, October 25th

Efforts Toward the Synthesis of α-Glucosidase Inhibitor Daphnepapytone A

James B. Martinez and Paul R. Hanson

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

More than 400 million people suffer from diabetes worldwide, and nearly 95% of those are afflicted with type 2 diabetes. Current treatments include α-glucosidase inhibitors such as acarbose, but their use is limited due to adverse side-effects and lack of potency. Daphnepapytone A is a sesquiterpenoid metabolite produced by the plant *Daphne papyracea* and is a more potent inhibitor of α-glucosidase than acarbose. While preliminary studies have shown potential for daphnepapytone A as a new chemotype for α-glucosidase inhibition, further testing has been hindered by low extraction yields. This project aims to synthesize daphnepapytone A chemically, enabling a more scalable production by circumventing the need for plant extraction. Biosynthetically, the unique tetracyclic cage structure of daphepapytone A has been proposed to originate from an oxidized guaiane intermediate, and two routes starting from (*R*)-carvone are investigated to validate this hypothesis. The azulene core is formed through either an allene-yne Pauson-Khand reaction or a santonin-like photorearrangement. A photochemical [2+2] cycloaddition is used to form the fused cyclobutane moiety found in the natural product.

Characterization of TolC Colicin Interactions

Andrew Daufel[1], Jimmy Budiardjo[1], Emre Firlar[3], Alex Bowman[1], Jason T. Kaelber[3,4], Joanna 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

Antibiotic resistance poses a threat to the human health gains made over the past century. In *Escherichia coli* some antibiotics are removed from the cell by efflux pumps. In Gram negative bacteria, they typically consist of a inner membrane component, a periplasmic component and an outer membrane component. In *E. Coli,* TolC is a trimeric outer membrane beta barrel, which adapts to multiple efflux pumps allowing small molecules to be removed from the cell. Given this conservation, if we could plug TolC, we could then potentially combat multiple different efflux pumps. Nature has also seen TolCs possibility for cellular access, and evolved proteins which bind into TolC. Colicins are *E. coli* proteins which use TolC and other outer membrane proteins to deliver cytotoxic payloads to other cells. Colicins, consist of an N-terminal translocation domain (T), a receptor binding domain (R), and a C-terminal cytotoxic domain (C). Several colicins bind TolC via their T-domains to facilitate the translocation of the toxic C-domain into the cell. We already identified that a colicin, Colicin E1 (ColE1), TR is able to reduce efflux mediated resistance. Additionally, we identified three TolC binding colicins, known as ColE1, Colicin 5 (Col5), and Colicin E1* (ColE1*). With our collaborators at Rutgers CryoEM center, along with biophysical characterization we have begun to understand some of the important factors of this interaction. We believe that a continued better understanding this interaction, could yield an effective TolC mediated efflux plug.

Exploration and Analysis of the Effects of Hydrogen Bonding on the Basicity of Mn^{III}-hydroxo Complexes in Proton-Coupled Electron Transfer (PCET) Reactions

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

[1]Department of Chemistry and Center for Environmentally Beneficial Catalysis, The University of Kansas, Lawrence, KS, USA; [2]J. Heyrovský Institute of Physical Chemistry, The Czech Academy of Sciences, Dolejškova 3, Prague 8, Czech Republic

Mn^{III}-hydroxo complexes are key intermediates in the metalloenzymes manganese lipoxygenase and manganese superoxide dismutase, where proton-coupled electron transfer reactions occur using a $Mn^{III}OH/Mn^{II}OH_2$ couple. In the active sites of these enzymes, there are amino acids that are capable of hydrogen bonding with the hydroxo ligand. Recently, we used two synthetic Mn^{III}-hydroxo model complexes to reveal that hydrogen bonding influences the rate of reactions between the Mn^{III}-hydroxo complexes and phenolic substrates in PCET reactions. In this work, we assess the role of hydrogen bonding as it relates to the basicity of the hydroxo ligand and explore how this changes PCET reactivity. Using experimental and computational methods, we were able to gain information about the thermodynamic properties of the reactions with different substrates. For the more acidic substrates, the rates fall outside of the trend, which we propose corresponds to a change in mechanism.

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

Erick McCloskey[1], Maithri Kashipathy[2], Anne Cooper[3], Philip Gao[3], David K. Johnson[4], Kevin P. Battaile[5], Scott Lovell[2], and David J. Davido[1]

[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; [2]Protein Structure and X-Ray Crystallography Laboratory, University of Kansas, Lawrence, KS, USA; [3]Protein Production Group, University of Kansas, Lawrence, KS, USA; [4]Computational Chemical Biology Core, University of Kansas, Lawrence, KS, USA; [5]NYX, New York Structural Biology Center, Upton, New York, USA

Infected cell protein 0 (ICP0) is an immediate-early regulatory protein of herpes simplex virus 1 (HSV-1) that possesses E3 ubiquitin ligase activity. ICP0 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 relates to these functions. ICP0 was purified and analyzed by X-ray crystallography, revealing a composition of nine β-sheets and two α-helices. Two neighboring β-sheets from each monomer "reach" into the adjacent subunit during dimer formation, generating two β-barrel-like motifs. Structural protein database searches indicate the fold/structure is novel, and the dimer is held together by an extensive network of hydrogen bonds. This structure allowed for the identification of residues involved in dimer formation. These residues were substituted to alanine to prevent dimerization, with the goal of preserving other functional domains in ICP0's C-terminus. Preliminary data from promoter activation assays suggest ICP0 dimer mutants are unable to effectively stimulate an HSV-1 promoter to the same degree as wild-type (WT) ICP0, and are unable to fully complement the replication of an ICP0-null mutant. Interestingly, these mutant forms of ICP0 show increased stability during infection compared to WT ICP0. Lastly, an 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. Future studies will determine how ICP0 dimerization regulates its functions and the HSV-1 life cycle.

Addressing Racial Disparities in CA125 Detection Rates Using a "Multi-omics" Approach

Ashten L. Gentry[1,2], Chien-Wei Wang[1,2], Rebecca J. Whelan[1,2]

[1]Department of Chemistry, The University of Kansas, Lawrence, KS, USA; [2]Ralph N. Adams Institute for Bioanalytical Chemistry, University of Kansas, Lawrence, KS, USA

The clinical assay for ovarian cancer biomarker CA125 operates on a "one-size-fits-all" approach, despite previous evidence showing that white patients tend to have higher levels of CA125 in serum when compared to Black patients. Since CA125 measured in serum is considered for a formal ovarian cancer diagnosis, these restrictive criteria lead to Black patients receiving more false negative diagnoses, and to higher fatality rates among Black patients than their white counterparts. We have proposed two non-mutually exclusive hypotheses for the observed disparity in serum CA125 levels: there is a difference in the amount of MUC16, the protein that bears the CA125 epitope, present in serum, or the MUC16 present in serum is structurally different and cannot be detected via immunoassay, due to the lack of the CA125 epitope. To test the first hypothesis, we will quantify MUC16 using a novel Parallel Reaction Monitoring (PRM) Mass Spectrometry assay, which will analyze patient plasma samples after Size Exclusion Chromatography (SEC) enrichment. To determine if there are structural differences in MUC16 found in the plasma of white patients and Black patients, we will sequence mRNA isolated from patient tumor samples via Oxford Nanopore. In this presentation, we report the progress on both of these goals, including mRNA isolation results, peptide selection for PRM development, and generation of an internal standard to enable quantitation.

Special Seminar – Saturday, October 26th

Tyrrell Conway, PhD

Regents Professor of Microbiology and Molecular Genetics, Principal Investigator and Director of Oklahoma Center for Microbiome Research - COBRE

Department of Microbiology and Molecular Genetics

Oklahoma State University

Oklahoma, OK, 74078

Nitrogen Assimilation by E. coli in the Mouse Intestine

Science component: Nitrogen is an essential element for all living organisms, including *Escherichia coli*. Potential nitrogen sources are abundant in the intestine, but knowledge of those used specifically by *E. coli* to colonize remains limited. Here, we sought to determine the specific nitrogen sources used by *E. coli* to colonize the streptomycin-treated mouse intestine. We began by investigating whether nitrogen is limiting in the intestine. The NtrBC two component system upregulates approximately 100 genes in response to nitrogen limitation. We showed that NtrBC is crucial for *E. coli* colonization, although most genes of the NtrBC regulon are not induced, which indicates that nitrogen is not limiting in the intestine. RNA-seq identified upregulated genes in colonized *E. coli* involved in transport and catabolism of seven amino acids, dipeptides and tripeptides, purines, pyrimidines, urea, and ethanolamine. Competitive colonization experiments revealed that L-serine, N-acetylneuraminic acid, N-acetylglucosamine, and di- and tri-peptides serve as nitrogen sources for *E. coli* in the intestine. Furthermore, the colonization defect of a L-serine deaminase mutant was rescued by excess nitrogen in the drinking water but not by an excess of a carbon and energy, demonstrating that L-serine serves primarily as a nitrogen source. Similar rescue experiments showed that N-acetylneuraminic acid serves as both a carbon and nitrogen source. To a minor extent aspartate and ammonia also serve as nitrogen sources. Overall, these findings demonstrate that *E. coli* utilizes multiple nitrogen sources for successful colonization of the mouse intestine, the most important of which is L-serine.

A brief overview of the Oklahoma Center for Microbiome Research – COBRE: The OCMR supports five research projects and an Anaerobic Microbiology Core Facility. The Anaerobic Core provides services for anaerobic culturomics to OCMR members and the scientific community across Oklahoma.

Financial support for the Center is provided by a *National Institute of General Medical Sciences CoBRE award (P20GM152333)*.

Invited Speakers, Saturday October 26th

Bacterial Competition in the Aerodigestive Tract: Antibiotics, Probiotics, and Beyond

Reed M. Stubbendieck

Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK, USA

The human nasal cavity is dominated by members of the phyla *Actinobacteria*, *Firmicutes*, and, in youth, *Proteobacteria*. To successfully colonize the nasal cavity, these bacteria must compete for scarce resources. This competition may occur indirectly by nutrient sequestration or directly through production of bioactive secondary metabolites or other antimicrobial effectors. In this talk, I will present two completed projects centered on competitive interactions in the nasal cavity mediated by *Actinobacteria*. In the first project, we found that *Rothia* were more abundant in the noses of healthy children than in sick children colonized by the pathogen *Moraxella catarrhalis*. We determined that *Rothia dentocariosa* and *Rothia similmucilaginosa* were able to fully inhibit the growth of *M. catarrhalis* in *in vitro* coculture assays and reduced M. catarrhalis loads in a respiratory air-liquid interface cell culture model. Using a combination of comparative genomics and proteomics, we identified a putative peptidoglycan hydrolase called secreted antigen A (SagA) produced by inhibitory *R. dentocariosa* and *R. similmucilaginosa*. We purified SagA and demonstrated that this enzyme inhibits *M. catarrhalis* growth and degrades peptidoglycan *in vitro*. In the second project, we determined that *Corynebacterium propinquum* produces an iron-chelating siderophore called dehydroxynocardamine. We found that production of this siderophore correlated with the ability of *C. propinquum* to inhibit the growth of coagulase-negative staphylococci *in vitro* and iron supplementation rescued staphylococci from inhibition. Furthermore, we found that the genes required for dehydroxynocardamine biosynthesis are expressed *in vivo*. These findings indicate that bacteria may produce siderophores to compete for limited iron in the human nasal cavity. I will end by discussing two recent projects in the laboratory focused on the protective role that the mutualist *Dolosigranulum pigrum* in the nose and the role that secondary metabolites play in mediating interspecies interactions in the lower airways of people with cystic fibrosis. Together, these projects demonstrate the capacity of commensal bacteria to protect against pathogen colonization in the human aerodigestive tract.

Sub-second Electrochemical Measurements of Oxytocin in Live Zebrafish Brains: Implications for Nociception

Michael A. Johnson

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

Zebrafish (*Danio rerio*) is a model organism first employed at the University of Oregon for the study of development. However, this organism is gaining recognition as a valuable tool to study neuronal function. Zebrafish have proven useful in understanding the role of oxytocin, a peptide hormone with numerous biological functions, in pain. We have developed a way to measure oxytocin with fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes, a method originally used to measure catecholamines in the brain. We optimized the waveform applied to the microelectrode to maximize the electrochemical response to oxytocin. We then used this method to measure optogenetically stimulated oxytocin release in whole, live brains from zebrafish. Next, we conducted an optogenetic/optochemical approach in which we investigated oxytocin release in zebrafish larvae in response to activation of TRPA1, which serves as a membrane-bound sensor for pain, cold, and itch in humans. Our results suggest that activation of TRP1 may act through the release of oxytocin onto spiny projection neuron cells.

Harnessing the Power of Polymer-enzyme Assemblies for Multimodal Cancer Therapy

Ming Zhao

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

Although various therapeutic modalities have been developed for cancer treatment, their therapeutic benefits are often greatly limited because of the pathophysiological tumor microenvironment (TME). Metabolic dysregulation, a hallmark of cancer, can be targeted with enzymes for metabolic interventions. In my presentation, I will introduce two polymer-enzyme nanoassemblies, designed for targeted delivery of enzymes and associated cancer drugs for TME modulation and combination cancer therapy.

The first nanoassembly consists of a second near-infrared (NIR-II) light-activated semiconducting polymer nanocore (SPN), a programmed death-ligand 1 (PD-L1) inhibitor, a nitric oxide (NO) precursor (L-arginine), and a thermoresponsive shell modified with the protease bromelain. Upon NIR-II light activation, bromelain is released to degrade tumor extracellular matrix, while SPN-induced cell death and NO-based gas therapy enhance tumor immunogenicity. Additionally, PD-L1 inhibition reverses the immunosuppressive TME, further promoting antitumor immune responses.

The second nanoassembly integrates an enzymatic cascade of glucose oxidase (Gox) and catalase (CAT) within a polymeric coating enriched with pH-responsive (hexamethyleneimine) (C7A) moieties. The nanocascade achieves effective tumor targeting, improves oxygenation within the tumor, and enhances radiosensitization, resulting in potent antitumor effects. These studies demonstrate versatile platforms for the precise and effective treatment of cancer.

Diffusible Molecules Produced by Skin Commensals and their Role in Colonization Resistance.

Rosana Ferreira

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

The human microbiota plays a crucial role in health and disease, with much of the research focusing on the gut. However, the role of the skin microbiota as a primary defense against pathogens remains less understood. *Cutibacterium acnes* and *Staphylococcus epidermidis* are among the most abundant skin commensals. Our laboratory is interested in investigating the role of these commensals on skin microbiome establishment and colonization by pathogens, specifically *Staphylococcus aureus*. Our work has revealed that molecules produced by these two commensals significantly reduce *Staphylococcus* biofilm formation, increase biofilm dispersion, and affect virulence gene expression. We have also determined that these molecules impact *Staphylococcus'* ability to adhere to and invade host epithelial cells, as well as determined their impact on cell viability and toxicity. Furthermore, we have made significant progress in identifying the bioactive molecules through High Performance Liquid Chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS). Our findings highlight the potential of bacteria isolated from the skin microbiome to produce molecules that affect *Staphylococcus'* ability to colonize and infect the host. This work highlights the importance of further exploring the skin microbiota for therapeutic applications.

Azole Drug Resistance in *Nakaseomyces glabratus*

Scott Moye-Rowley[1] and **Bao Vu[2]**

[1]*Department of Molecular Physiology and Biophysics, Carver College of Medicine University of Iowa, Iowa City, IA, USA*; [2]*Department of Microbiology and Immunology, University of Oklahoma Health Sciences, Oklahoma City, OK, USA.*

Antifungal azole drugs inhibit the biosynthesis of ergosterol, which is vital for the fungal plasma membrane function. To compensate for the azole inhibitory effect, *Nakaseomyces glabratus* (formerly named *Candida glabrata*) upregulates the expression of ergosterol synthesis genes and activates the multidrug transporter system. Our studies have indicated that the transcription factor Upc2A functions as a physiological link between the ergosterol synthesis and the multidrug transporter systems, by cross-regulating genes in both pathways. In addition, Upc2A function is essential for *N. glabratus* hypoxic growth, and hypoxia significantly enhances azole efficacy. These findings have provided new directions in understanding drug resistance mechanisms in *N. glabratus*, and presented Upc2A as a potential target for antifungal development.

CYP5122A1 Encodes an Essential Sterol C4-methyl Oxidase in *Leishmania donovani* and Determines the Antileishmanial Activity of Antifungal Azoles

Michael Zhuo Wang, Ph.D.

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

Visceral leishmaniasis is a life-threatening parasitic disease, but current antileishmanial drugs have severe drawbacks. Antifungal azoles inhibit the activity of cytochrome P450 (CYP) 51 enzymes which are responsible for removing the C14α-methyl group of lanosterol, a key step in ergosterol biosynthesis in *Leishmania*. However, they exhibit varying degrees of antileishmanial activities in culture, suggesting the existence of unrecognized molecular targets. Our previous study reveals that, in *Leishmania*, lanosterol undergoes parallel C4- and C14-demethylation to form 4α,14α-dimethylzymosterol and T-MAS, respectively. In the current study, CYP5122A1 is identified as a sterol C4-methyl oxidase that catalyzes the sequential oxidation of lanosterol to form C4-oxidation metabolites. CYP5122A1 is essential for both *L. donovani* promastigotes in culture and intracellular amastigotes in infected mice. CYP5122A1 overexpression results in growth delay, increased tolerance to stress, and altered expression of lipophosphoglycan and proteophosphoglycan. CYP5122A1 also helps to determine the antileishmanial effect of antifungal azoles *in vitro*. Dual inhibitors of CYP51 and CYP5122A1 possess superior antileishmanial activity against *L. donovani* promastigotes whereas CYP51-selective inhibitors have little effect on promastigote growth. Our findings uncover the critical biochemical and biological role of CYP5122A1 in *L. donovani* and provide an important foundation for developing new antileishmanial drugs by targeting both CYP enzymes.

Profiling DNA-Protein Crosslinks

Luke Erber

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

Crosslinking between DNA and protein results in a bulky DNA lesion that distorts the DNA helix and obstructs essential DNA-protein interactions necessary for DNA replication, transcription, repair, recombination, and chromatin remodeling. DNA-protein crosslinks (DPCs) can be induced by exposure to endogenous metabolites, exogenous agents and chemotherapies like platinum drugs. If not repaired, DPCs lead to permanent DNA alterations and toxicity. DPCs formed by endogenous metabolites are elevated in individuals suffering from Ruijs-Aalfs syndrome, a genetic disorder defined by polymorphisms in the SPRTN gene, encoding a protease involved in DPC repair. Patients with Ruijs-Aalfs exhibit genomic instability, premature aging, and develop hepatocellular carcinoma. My recent

studies have focused on developing tools to uncover the structures of DNA-protein crosslinks induced by various endogenous cross-linking agents and determine repair mechanisms. In the present work, we quantified DPC formation in human cells exposed to elevated levels of methylglyoxal (MGO), an electrophilic *α*-oxoaldehyde generated endogenously through metabolism of carbohydrates. We identified proteins trapped on DNA upon MGO exposure using mass spectrometry-based proteomics. Collectively, this study provides the first evidence for MGO-mediated DNA–protein cross-linking in living cells, prompting future studies regarding the relevance of these toxic lesions in cancer, diabetes, and other diseases linked to elevated MGO levels

Neuro-immune-microbe Interactions in the Gut

Hongbing Yu

Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, KS, USA

Our gut immune system has >70% of the body's immune cells. It constantly interacts with diverse microbes (non-pathogenic and pathogenic) and nutrients in the gut lumen to regulate health in the gut and other distal organs. Emerging evidence indicates that the gut immune system also communicates with the enteric nervous system (ENS) to coordinate host responses to 15uminal contents. In this talk, I will discuss how a neuropeptide named vasoactive intestinal peptide (VIP), secreted by the ENS, regulates the recruitment of immune cells to the gut, and how this affects the host's susceptibility to enteric pathogen infections. A deeper understanding of molecular and cellular mechanisms involved in these neuro-immune-microbe interactions should help us to develop better strategies targeting microbes and/or host respsonses in infectious and immune diseases.

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

Chamani T. 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 encompasses the Purification and Analysis Laboratory (PAL) that provides purification, analysis and quality control of compounds via LC/MS. The SCB core also provides MALDI-TOF analysis of biomolecules.

How to discover bioactive small molecules (when all you have is synthetic chemistry and cell culture)?

Zarko Boskovic

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

My talk is focused on the central problem of applied synthesis, and that is discovering the relationship between chemical structures and their properties or activity. I will describe the problem by using matrix algebra terminology, and I will demonstrate how structural complexity of small organic molecules can be a useful metric for unbiased exploration of new chemical space. I will then provide several examples

of the chemistry we developed, as well as some early insights into what these biologically naïve molecules may be doing in complex cellular setting.

Posters

#1 – RpoN-Dependent Phosphotransferase Systems in *Enterococcus faecalis*

Tolulope I. Ade, Christian D. Decker and Lynn E. Hancock

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

Phosphotransferase systems (PTS) play significant roles in the uptake and phosphorylation of sugars for metabolism. *Enterococcus faecalis* is predicted to encode 46 distinct PTS pathways, highlighting its versatility to grow on a variety of carbon sources, but many of the PTS substratesare unknown. In *E. faecalis*, six PTS are predicted to be regulated by the alternative sigma factor,RpoN, but only a small number of PTS substrates are known. To identify potential substrates for the RpoN-dependent PTS, we performed a Biolog carbon source phenotype array comparing commonly used strains of *E. faecalis* along with their isogenic *rpoN* mutants. We also took a bioinformatic approach to identify functionally characterized PTS from other organisms that showrelatedness to the *E. faecalis* RpoN-dependent PTS. RpoN-dependent gene regulation in *E. faecalis*is also dependent on five bacterial enhancer binding proteins (bEBPs) of the LevR-family. Biologresults showed that the metabolism of glucose, mannose, cellobiose, gentiobiose, arbutin, salicin,glucosamine, and amygdalin require PTSs that are RpoN-dependent. Through bioinformatics, wewere able to identify additional sugar substrates (glucosaminic acid, glucoselysine and fructoselysine) as potential PTS substrates dependent on RpoN. Through mutational analysis of the various bEBPs in *E. faecalis*, we were able to demonstrate a linkage between those sugars anda dedicated PTS responsible for their import. We also demonstrate through luciferase reporter assays that the PTS operons are induced by the sugar substrates in a manner that requires both RpoN and the corresponding bEBP.

#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) that converts polyunsaturated fatty acids to alkyl hydroperoxides in fungi and plants, which allows these substrates to be further metabolized. In the suggested reaction mechanism for this enzyme, transfer of a hydrogen atom from the substrate C-H bond to a Mn^{III}-hydroxo center (active site) starts the substrate oxidation. Inspired by these enzymes, scientists have synthesized models complexes of the enzyme active site. These complexes have been shown to activate hydrogen peroxide to conduct C-H bond activation, olefin epoxidation and sulfoxidation with high selectivity. It is proposed that the key intermediate of these Mn complexes and enzymes is a Mn^{V}-oxo generated by the breaking of O-O bond in a Mn^{III}-hydroperoxo complex. A new Mn^{III}-hydroperoxo complex ($[Mn^{III}(OOH)(^{6Me}dpaq)]^{+}$) was formed by reacting a Mn^{III}-hydroxo, supported by a $dpaq^{6Me}$ ligand (an amide-containing, pentadentate ligand), with H_2O_2 and $HClO_4$ which was found to be stable at room temperature. This intermediate could do oxygen and hydrogen atom transfer. According to kinetic studies, this new intermediate reacts faster with substrates than analogous MnIII-hydroperoxo and MnIII-alkylperoxo complexes. A comparison of $[Mn^{III}(OOH)(^{6Me}dpaq)]^{+}$ with similar MnIII-hydroperoxo and MnIII-alkylperoxo complexes was conducted using Time-dependent computations (TD-DFT) to understand how the differences in the ligand sphere can affect the electronic structures of the complexes.

#3 – Decellularization of Animal Organs as a Platform for Tissue Engineering Disease Modeling

Connor Ahlquist[1,2] and Hyunjoon Kim[1,2]

[1]*Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS, USA;*
[2]*Bioengineering Program, The University of Kansas, Lawrence, KS, USA*

The extracellular matrix (ECM) has historically been researched as a tissue engineering scaffold and characterization platform elucidating the role of matrix protein complexes in native and diseased states. Various studies have reported the decellularization of animal organs to isolate their extracellular matrix; however, due to the varying complexities of tissue niches between animal types and specific organs, a single, comprehensive protocol has yet to be developed. Therefore, a lab-specific decellularization protocol is required to integrate an extracellular matrix platform into our research on delivery of therapeutics for the treatment of bladder cancer. Identifying the altered, overexpressed, or unique protein complexes within the bladder ECM can elucidate novel delivery and therapeutic targets to treat non-invasive and invasive bladder cancer. A comparison of decellularization efficacy of protocols was performed utilizing both chemical and physical decellularization techniques. Sodium Dodecyl Sulfate (SDS), Triton X-100, and ammonium hydroxide (NH_4OH) were selected as competent chemical decellularization agents due to their ability to disrupt and denature the cellular membrane while preserving native protein structures in the ECM. These agents were supplemented with a freeze and thaw cycle (FTC) to promote the lysing of the cellular membrane and therefore enhance the removal of cells from the tissue. Here I report a comparison between literature derived decellularization protocols and their application in various animal and tissue types in which a significant removal of cells was observed via DNA quantitation in murine stomach, small intestine, and bladder as well as porcine bladder samples. Through analysis of total DNA concentration, a combinatorial protocol utilizing FTC, SDS, Triton X-100, and NH_4OH showed the most effective in the removal of cellular content in specific tissue niches.

#4 – Identification of Genes that Contribute to Infection Processes of *Chlamydia* during Animal Infection

Lauren Atkinson and P. Scott Hefty

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

Chlamydia is the most common sexually transmitted bacterial infection and the leading cause of infectious blindness worldwide. Few gene products have been experimentally determined to be important for infection and disease processes, and many of the mechanisms for its basic biology are poorly understood. Moreover, a relatively large proportion of most *Chlamydia* genomes (25-40%) encodes hypothetical proteins of unknown functions. To identify gene products important to either its basic biology or infection processes, members of a *C. muridarum* transposon mutant library were evaluated in a murine infection model as well as for their growth characteristics in cell culture. Of the sixteen strains tested, five exhibited an impaired ability to ascend from the vaginal vault to the upper reproductive tract. Analysis in cell culture supported a defect in basic growth aspects for four of these five mutants. Two of these strains contain disruptions of genes encoding chlamydial-specific conserved hypothetical proteins. Together, these data support the identification of two protein of unknown function that are important to the basic growth of *Chlamydia* and ascension to the upper genital tract, a key aspect for *Chlamydia* pathogenesis.

#5 – Regulation of *cdeAB-oprM* Efflux Pump in *Chromobacterium subtsugae* in Response to Antibiotics and Quorum Sensing

Samalee Banerjee[1], Eryk Yarkosky[1], Nathan Smith[1] , Cassie Doody[2], Helen Blackwell[2], and Josephine R. Chandler[1]

[1]*Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA;* [2]*Department of Microbiology, University of Wisconsin-Madison, Madison, WI, USA*

Chromobacterium subtsugae is a non-pathogenic soil bacterium that has been used as a model to study bacterial interactions in mixed microbial communities. During interspecies competition *C. substugae* defends against antibiotics produced by other species using the CdeAB-OprM efflux pump, which transports antibiotics and other substrates out of the cell. We previously showed the *cdeAB-oprM* genes are activated by the CviR-CviI quorum-sensing system, which senses and responds to changes in population density via a CviI-produced chemical signaling molecule (acyl-homoserine lactone, or AHL) and the AHL-responsive transcriptional regulator CviR. The *cdeAB-oprM* genes are also repressed by an antibiotic-responsive TetR-family transcription factor, CdeR. In this study we examined potential interactions between CviR and CdeR and the specific mechanisms of each regulator. We constructed strains with deletion mutations in each regulator gene and showed that both CviR and CdeR can regulate the *cdeAB-oprM* genes independent of the other regulator. We also carried out in vitro promoter binding experiments and showed that purified CviR does not directly interact with the *cdeA* promoter, suggesting CviR regulation is likely through indirect effects on other (unknown) regulators. We identified a potential CdeR binding site in the *cdeA* promoter based on conservation with the binding sites of other structurally related TetR-family proteins. Single-base mutations in this site abolished CdeR repression, supporting the idea that CdeR interacts directly with the *cdeA* promoter through this site. We also identified potential CdeR binding sites in the promoters of a handful of other genes in the genome and experiments are underway to test the hypothesis that CdeR is a global, not local, regulator of antibiotic defense responses. These results support a model the *cdeAB-oprM* genes are independently regulated in response to different environmental cues (antibiotics and population density), which may have certain benefits during antibiotic defense. These findings contribute to a better picture of how bacteria coordinate the regulation of antibiotic defense mechanisms during competition with other species.

#6 – *Cutibacterium acnes* Molecules Reduce Mature Biofilms of Different *Staphylococcus* Species

Olivia R. Bauer, Rayssa D. Lima, Rosana B. Ferreira

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

Staphylococcus species are frequently isolated from human and animal infections, both in hospitals and in the community. The rapid spread of antimicrobial resistance among these species indicates the importance of discovering new alternatives for the treatment of such infections. Biofilm formation is an important way in which these species establish and persist in the host and the environment. Infections associated with biofilms are more difficult to treat, highlighting the importance of compounds capable of acting on these structures. *Cutibacterium acnes*, an important member of the human skin microbiome was shown to protect the skin against certain pathogens. In previous work, we investigated whether molecules produced by *C. acnes* could affect the biofilm formation of *Staphylococcus* species. We have seen that these molecules have antibiofilm activity against *S. lugdunensis* without affecting its planktonic growth. In the present study, we investigated whether *C. acnes*-produced molecules have activity against mature biofilms of different *Staphylococcus* species. To do this, *C. acnes* is grown in an anaerobic chamber, and its supernatant is collected, filtered, and concentrated to obtain cell-free conditioned media (CFCM). *Staphylococcus* strains are grown in biofilm assays on polystyrene plates for 24 hours at 37ºC. After, mature biofilms are incubated with different concentrations of CFCM or vehicle (PBS) for another 24 hours at 37ºC. We observed that *C. acnes* CFCM reduces *S. aureus, S. epidermidis, S. hominis* and *S. pseudintermedius* mature biofilm mass. Interestingly, these molecules did not impact *S. lugdunensis* mature biofilm. Initial characterization of the bioactive molecules with activity against mature biofilm was performed and revealed that the molecules are resistant to boil

(100°C for 60 min) and smaller than 3KDa. Next, we will determine if the molecules are resistant to treatment with proteinase K and sodium periodate. Investigating compounds that impact *Staphylococcus* biofilm structure might reveal potential applications of molecules produced by the skin microbiome.

#7 – The Autoimmunity-Associated Minor Allele of *PTPN22* Enhances Antiviral Immunity During Coronavirus Infection

Alec M. Bevis[1,2], Anam Shaikh[1,2], Catherine Kerr[1,2], Jenna Barnes[1], Kate Rosa[1], Tammy Cockerham[1], Nancy Schwarting[1], Anthony R. Fehr[1], Robin C. Orozco[1].

[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA;[2]The Office of Graduate Studies, University of Kansas, Lawrence, KS, USA

The deadly consequences of viral infection were clearly demonstrated by the COVID-19 pandemic. Therefore, identifying new therapeutic targets to enhance our antiviral immunity and combat virus infection is essential, such as the immune-regulatory gene *PTPN22*. 5-15% of North Americans express a *PTPN22* alternative allele which is linked with multiple autoimmune diseases. However, there is a significant research gap regarding the role of *PTPN22* and its common allele during viral infection. To address this, we used CRISPR/Cas9 generated *Ptpn22* knockout (PEP-null) and *Ptpn22* minor allele expressing (PEP-619WW) mice and infected them with the common murine model of coronavirus, Mouse Hepatitis Virus (MHV) A59. We hypothesize that PEP-null and PEP-619WW mice have enhanced antiviral immunity during coronavirus infection. Following MHV A59 infection, PEP-null and PEP-619WW mice have reduced weight loss and increased survival over WT mice. Additionally, PEP-null and PEP-619WW mice have enhanced innate immunity, such as increased Natural Killer (NK) cell numbers. Furthermore, we show that PEP-null and PEP-619WW innate immune cells enhance protection during MHV A59 infection, but lymphocytes are necessary for survival. These results demonstrate that PEP-null and PEP-619WW are beneficial during coronavirus infection. This research sets the precedent to interrogate the role of *Ptpn22* in other RNA virus infections and as a novel therapeutic target to enhance antiviral immunity.

#8 – ELD403 as a Novel Anti-Viral Therapy for RSV-related Infections in Cystic Fibrosis

Matthew Biggart, Flori Sassano, Robert Tarran

Division of Genetic and Environmental Inhalation Disease, Kansas University Medical Center, Kansas City, Kansas, USA

Background:

Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis in infants and a major cause of exacerbations in both patients with cystic fibrosis (CF) and the immunocompromised. In healthy individuals RSV typically causes a self-limiting infection with mild symptoms. However, in patients with CF ~45% of exacerbations are associated with respiratory viruses. Despite recent advances in creating an RSV vaccine, the last two seasons have been of note for their severity and high number of hospitalizations due to RSV, indicating a continued need for new therapeutics. We hypothesized that patients with CF may lack an antiviral defenses. We carried out proteomic analysis of CF and non-CF sputum and identified ELD403, which was present in non-CF sputum but absent from CF sputum. Here, we tested the hypothesis that ELD403 exhibited antiviral properties. To test this hypothesis, we studied ELD403's effects in CF and non-CF human bronchial epithelial cells.

Methods:

Direct interactions between ELD403 and RSV were examined using fluorescent microscopy, dynamic light scattering and transmission electron microscopy. Additionally, peptide aggregation of RSV was examined via isolation of aggregates, re-suspension and examination of the optical density at 380nm (OD_{380}). ELD403's antiviral properties were examined for therapeutic or prophylactic effects in both HEP2 cells, and primary CF and non-CF HBECs.

Results:

Treatment with 100 μM ELD403 lead to a significant increase in the size of dylight-633 labelled RSV (RSV-633) particles from 116 (±18) nm to 264(±39) nm (n=6, p=0.027) and a decrease in overall number of free particles from 585 (±321) to 60 (±32) (n=5, p=0.046). This was further confirmed on label-free RSV using dynamic light scattering and transmission electron microscopy. The addition of 100 μM ELD403 lead to a significant decrease in binding of the virus to proteins in the plasma membrane at 4˚C when compared to RSV-633 alone, shifting from 2011±741 relative fluorescent units (RFU) to 1238±182 RFU (n=6, p<0.001). Furthermore, this concentration reduced internalization of virus after 2 hours of exposure, decreasing intracellular fluorescence from 8204±460 RFU with RSV-633 alone to 1829±1545 RFU (n=4, p=0.028). OD_{380} scanning of resuspended aggregates showed that addition of ELD403 lead to a suspension with a significantly higher optical density when compared to the virus alone increasing from 0.0847(±0.0067) to 0.1223(±0.0040) OD_{380} (n=4, p=0.054). ELD403's antiviral effect showed greater potency on the inhibition of RSV infections as a prophylactic agent, providing an IC50 of 78.86 μM, 62.45 μM and 77.34 μM in cultures of CF, non-CF and HEP2 cells respectively. When the same cell types were provided a therapeutic course of ELD403 the IC50 increased to 209.20 μM, 124.70 μM and 79.48 μM respectively.

Conclusion:

ELD403 directly interacts with RSV causing the virus to aggregate. These larger viral aggregates are less able to bind and internalize, reducing the percentage of infected cells. Decreasing the free viral particle number through binding and aggregation provides a novel mechanism through which ELD403 acts as an anti-viral agent to RSV. This mechanism is being further explored through proteomic analysis of the RSV:ELD403 interaction. The lack of ELD403 could be a contributing factor to CF patients reduce viral clearance.

#9 – Role of Haspin kinase in Topoisomerase II Response Checkpoint (TRC) activation in Human Cells

Asbin Chand and Yoshiaki Azuma

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

Haploid germ cell-specific nuclear protein kinase (Haspin) is a conserved atypical eukaryotic serine-threonine kinase. Haspin phosphorylates Histone 3 threonine 3 (H3T3p); the only known target of Haspin. H3T3p recruits chromosome passenger complex (CPC) consisting of Aurora B kinase. Aurora B maintains genome stability by monitoring spindle assembly checkpoint to ensure correct kinetochore-microtubule (KT-MT) attachment and microtubule tension during early mitosis. DNA topoisomerase II (TopoII) and its unique strand-passage reaction (SPR) are crucial for faithful chromosome segregation. More importantly, TopoII is found to be involved in monitoring mitosis progression by activating a cell cycle checkpoint called TRC. The TRC is conserved among species. Recent studies have shown that TopoII SUMOylation is a key player behind the TRC activation. We have demonstrated that in *Xenopus laevis* egg extract catalytic inhibition of DNA topoisomerase II by ICRF-193 increases SUMOylation of its CTD. By utilizing SUMO interacting motifs (SIM), SUMOylated TopoII interacts with Haspin which

recruits the Aurora B through H3T3p at centromere. However, this molecular circuit has not been tested and confirmed in human cells.

Moreover, in human cells, Haspin inhibitors completely abolished ICRF-193-induced checkpoint activation but inhibition of TopoII SUMOylation showed partial bypass. However, the exact interactivity of mitotic Haspin and TopoII SUMOylation remains unclear. We will utilize the Auxin Inducible Degron (AID) system combined with the Tetracycline inducible expression (Tet-ON) system to explore possible mechanisms and interactions between TRC activation and the mitotic behavior of Haspin kinase.

#10 – Exonuclease Time of Flight Sensor for Single Molecule RNA Sequencing

Indu A. Chandrasoma[1,2], Farhad Shiri[1,2], Junseo Choi[2,7], Sunggook Park[2,7], Malgorzata A. Witek[1,2,5], and Steven A. Soper[1,2,3,4,5,6]

[1]Department of Chemistry, The University of Kansas, Lawrence, KS, USA; [2]Center of BioModular Multi-scale Systems for Precision Medicine, The University of Kansas, Lawrence, KS, USA; [3]Department of Cancer Biology, The University of Kansas Medical Center, Cancer Center, Kansas City, KS, USA; [4]Bioengineering Program, The University of Kansas, Lawrence, KS, USA; [5]Kansas Institute for Precision Medicine, University of Kansas Medical Center, Kansas City, KS, USA; [6]Department of Mechanical Engineering, The University of Kansas, Lawrence, KS, USA; [7]Mechanical Engineering, Louisiana State University, Baton Rouge, LA, USA

Transcriptomics is advancing disease diagnosis, prognosis, and treatment by uncovering RNA structure complexities, with single-molecule sequencing (SMS) technologies like Oxford Nanopore Technology (ONT) offering notable advantages over next-generation sequencing (NGS). ONT benefits from direct RNA sequencing without amplification, long sequence reads, label-free electrical detection, and a simplified workflow, although it requires substantial sample input (~1 μg). To address this, we are developing the X-ToF, a novel SMS nanofluidic device made from thermoplastic using nano-injection molding, which utilizes an immobilized enzyme, Exoribonuclease 1 (XRN1), to cleave single-stranded RNA into ribonucleotide monophosphates (rNMPs). The X-ToF detects, identifies individual rNMPs by measuring their specific time-of-flight (ToF) or electrophoretic mobility using Resistive Pulse Sensing (RPS), significantly reducing sequencing costs, simplifying library preparation, and enhancing read accuracy. This device comprises a nanofluidic circuit with a solid-phase bioreactor where XRN1 is covalently immobilized to cleave RNA, followed by a nanochannel with dual in-plane nanopore sensors to determine the ToF of each rNMP accurately. In this presentation, we report on utilizing the X-ToF sensor to collect multiple parameters beyond time-of-flight (ToF) for our measurement scheme, such as pore 1 dwell time, pore 2 dwell time, pore 1 peak amplitude, and pore 2 peak amplitude of each rNMP to create a comprehensive library for future RNA sequencing. We demonstrate an 95.11% identification accuracy using Principal Component Analysis (PCA) as an unsupervised machine learning technique, achieving 100% accuracy with neural networks as a supervised approach. Additionally, we show the capability to sequence a single RNA molecule within the X-ToF device's bioreactor by reducing the RNA input requirement to 10 pM. The XRN1 enzyme's clipping rate was confirmed as 25 ± 6 nts/s by introducing a new measurement modality, interpeak pair space. With these insights, we have initiated RNA sequencing and aim to increase the alignment percentage of RNA in future work.

#11 – CD8+ T Cells Are Not The Key Drivers of Weight Loss in PEP-619WW Mice During Chronic Virus Infection

Mollie Coffey[1], Robin C. Orozco[2]

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

Immune cells can drive pathology during virus infections. For example, CD8+ T cells cause weight loss during LCMV-cl13, a chronic virus infection. Previously, we found that mice that express a common allelic variant of PTPN22 (PEP-619WW) have less weight loss and can clear the LCMV-cl13 infection. Also, PEP-619WW mice had enhanced T cell function. The dichotomy of more T cell function but less weight loss in PEP-619WW infected mice led us to question, what is causing the weight loss in these mice? To answer this, PEP-WT and PEP-619WW mice were treated with antibodies to deplete their CD8+ T cells and were infected with LCMV-cl13 and weights were measured. Notably, our results showed that CD8+ T cells did not mediate the weight loss in PEP-619WW mice. However, Rag1 KO PEP-619WW mice did not lose weight during infection. We also quantified the amount of virus in these animals. These data show that there is another driving factor other than CD8+ T cells mediating weight loss in PEP-619WW mice.

#12 – Characterization of TolC Colicin Interactions

Andrew Daufel[1], Jimmy Budiardjo[1], Emre Firlar[3], Alex Bowman[1], Jason T. Kaelber[3,4], Joanna 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

Antibiotic resistance poses a threat to the human health gains made over the past century. In *Escherichia coli* some antibiotics are removed from the cell by efflux pumps. In Gram negative bacteria, they typically consist of a inner membrane component, a periplasmic component and an outer membrane component. In *E. Coli,* TolC is a trimeric outer membrane beta barrel, which adapts to multiple efflux pumps allowing small molecules to be removed from the cell. Given this conservation, if we could plug TolC, we could then potentially combat multiple different efflux pumps. Nature has also seen TolCs possibility for cellular access, and evolved proteins which bind into TolC. Colicins are *E. coli* proteins which use TolC and other outer membrane proteins to deliver cytotoxic payloads to other cells. Colicins, consist of an N-terminal translocation domain (T), a receptor binding domain (R), and a C-terminal cytotoxic domain (C). Several colicins bind TolC via their T-domains to facilitate the translocation of the toxic C-domain into the cell. We already identified that a colicin, Colicin E1 (ColE1), TR is able to reduce efflux mediated resistance. Additionally, we identified three TolC binding colicins, known as ColE1, Colicin 5 (Col5), and Colicin E1* (ColE1*). With our collaborators at Rutgers CryoEM center, along with biophysical characterization we have begun to understand some of the important factors of this interaction. We believe that a continued better understanding this interaction, could yield an effective TolC mediated efflux plug.

#13 – Quantification of Central Nervous System Cholesterol and Cholesterol Metabolites during Demyelination and Remyelination in a Genetic Mouse Model

Hudihim MWDK Dedunupitiya[1]; Eden Parreno Go[1]; Nishama De Silva Mohotti[1]; Jenna Marie Williams[1]; Hiroko Kobayashi[1]; Rashmi Basavaraj Binjawadagi[1]; Heather Desaire[1]; Meredith Hartley[1]

[1]University of Kansas, Lawrence, KS, USA

Multiple sclerosis is caused by the inflammatory demyelination of the myelin sheath. Cholesterol is one of the significant lipids in the myelin. Our research group has observed high levels of cholesterol esters (CE) during demyelination, and it decreases upon remyelination in a genetic mouse model. This leads us to investigate more about molecular level fluctuations of cholesterol and cholesterol metabolites during demyelination and remyelination using mass spectrometry. Our first objective is to develop a method and apply this method to measure how CE levels are affected by the treatment of Sob-AM2 remyelinating drug in a genetic mouse model. A genetic mouse model based on the inducible, conditional ablation of the *Myrf* gene was used to produce demyelinating (*cre* +) and non-demyelinating (*cre* -) mouse groups. This analysis shows a significant difference between the demyelinating mice of Sob-AM2-treated and vehicle-treated groups for low-abundant CEs but not for the highly abundant CEs. Interestingly, all the highly abundant CEs are polyunsaturated. This method is currently being optimized for the quantification of CEs in microglial cell fractions.

The second research project is focused on the quantification of cholesterol and oxysterols during demyelination and remyelination in the brain and spinal cord using GC-MS. Lipids extracted from mouse brain and spinal cord tissue samples contains a very high level of cholesterol compared to other sterols, which limits the quantification of other sterols in the same run. Therefore, cholesterol and oxysterols were separated from other brain lipids using Isolute Si SPE cartridges. Separated cholesterol and oxysterol fractions were derivatized with trimethylsilylation for GC-MS. Cholesterol in the mouse brain was quantified as the very first step using internal standard calibration curves under the single ion monitoring method of the GC-MS. The total cholesterol level is increasing with age in healthy mouse brains, while the demyelinated mice group has no significant increase in cholesterol levels. Currently, mass spectrometric methods are being optimized to understand fluctuations of sterols and cholesterol metabolites during demyelination and remyelination in both brain and spinal cord samples. Mapping cholesterol and its metabolite regulation and its role in demyelination and remyelination would unveil a pathway of future potential therapeutic targets of remyelination.

#14 – Optimizing the Delivery of Antibody Therapeutics using Blood Brain Barrier Derived Peptides

Eric Ebert[1], Kelly Schwinghamer[1], Donald W. Miller[2], Teruna J. Siahaan[1]

[1]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, USA; [2]Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, MB, CA

Monoclonal antibody (mAb) therapeutics are an important class of drug 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) can increase the paracellular porosity of the BBB to allow the permeation of mAb into the brain. Previously, cadherin peptides (i.e., ADTC5, HAVN1) 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 BBB modulator (BBBM) peptides from VE-cadherin; (2) evaluate the safety of repeated use of BBBM peptides *in vivo*; and (3) evaluate the therapeutic efficacy of BDNF peptides when co-delivered with BBBM peptides in the APP/PS1 AD mouse model. 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. We also plan to investigate the brain deposition of mAbs using positron emission tomography. Finally, the efficacy of co-administration of BDNF peptide(s) and BBBM peptides to improve cognitive behavior and neuroregeneration in the APP/PS1 AD mouse model will be evaluated.

#15 – Evaluation of a Novel Toll-like Receptor Agonist for Nanoparticle-based Immunotherapy

Grahmm A. Funk, Hyunjoon Kim

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

Purpose: Immunotherapy serves to help activate the host immune system to fight foreign pathogens or cancers. These therapies often include the use of small molecule immunostimulants such as toll-like receptor (TLR) agonists that interact with immune cells which can trigger a robust immune response through the production of proinflammatory cytokines and changes in immune cell-regulating surface marker expression. Conventional immunotherapies utilize parenteral injections or intravenous infusions of the free drug. Unfortunately, soluble drugs are rapidly cleared from the administration site and circulation, limiting their interaction with the desired target. The use of drug-encapsulating nanoparticles, such as poly(lactic-co-glycolic) (PLGA)-based nanoparticles, enable superior cellular uptake and delivery of small molecule-based immunotherapies. This study aims to evaluate the immunostimulatory activity of a novel toll-like receptor 7/8 agonist (TLR8). Furthermore, we evaluate the characteristics of two PLGA nanoparticle formulations as TLR8 carriers to enhance cellular uptake and create a more potent immunotherapy.

Methods: *In vitro* immunostimulatory activity of TLR8 was evaluated against murine bone marrow-derived dendritic cells (BMDCs) and splenocytes. Harvested cells were treated with either 100 nM, 1 mM, or 10 mM TLRa for 48 hours, with a known TLR7/8 agonist, Resiquimod (RESQ), being used as a comparative control. Both IL-10 and TNF-α cytokine secretion into the cell media was quantified via ELISA. Immunophenotyping was performed by flow cytometry analysis. Two different nanoparticle formulations were employed: A pure PLGA formulation using a solvent evaporation method, and a PLGA:PLGA-PEG (polyethylene glycol) formulation using a nanoprecipitation method. Hydrodynamic radius, polydispersity index (PI), and zeta potential (surface charge) of both TLR8-loaded and unloaded nanoparticles was measured via dynamic light scattering (DLS). Cellular uptake of fluorescent coumarin-6-loaded nanoparticles was performed using Caco-2 cells and imaged using a fluorescent microscope.

Results: TLR8-stimulated BMDCs expressed high amounts of both IL-10 and TNF-α cytokines, on par with RESQ positive control. TLR8 increased the surface expression of CD40, CD80, and CD86 on BMDCs significantly above media controls. TLR8 also increased the expression of CD69, INF-γ, and TNF-α in splenocytes following treatment, with CD69 expression being superior to that of RESQ-treated splenocytes. Both nanoparticle formulations were able to successfully encapsulate TLR8. DLS data revealed PLGA-PEG nanoparticles to have a smaller size and higher surface charge.

Conclusions: Initial discovery of potential new immunomodulatory drugs is crucial to develop increasingly potent and targeted immunotherapies. The ability of TLR8 to increase immune-regulating cytokine production and cell surface marker expression enables this compound to be used for immunotherapy regimes. Successful incorporation into nanoparticles will allow for greater cellular uptake and potency *in vivo*.

Acknowledgement: Personnel funding (G.A.F) provided by the Madison and Lila Self Graduate Fellowship.

#16 – Equipment and Services of the Kansas University Nanofabrication Facility

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

[1]The Center for Molecular Analysis of Disease Pathways, University of Kansas, Lawrence, KS, USA; [2]The Ralph N. Adams Institute for Bioanalytical Chemistry, University of Kansas, Lawrence, KS, USA; [3]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, USA; [4]Department of Chemistry, 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, metal and dielectric material thin film deposition, scanning electron microscopy (VP-SEM), atomic force microscopy, contact angle goniometry, ellipsometry, profilometry, wafer dicing, 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, dedicated process fume hoods and filtered lighting for photolithography.

This facility is under the direction of Dr. Susan Lunte. 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.

#17 – 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 already 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/.

#18 – Commensal *Staphylococcus epidermidis*-diffusible Molecules affects *Staphylococcus aureus* Virulence

Kiana Hajiarbabi, Ankur Sood, Rayssa D. Lima, Nathalia S. Magalhães and Rosana B. R. Ferreira

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

The human microbiota plays a crucial role in health and disease, with much of the research focusing on the gut. However, the role of the skin microbiota as a primary defense against pathogens remains less understood. Our previous findings revealed that molecules present in the cell-free culture medium (CFCM) of commensal *Staphylococcus epidermidis* significantly reduce *Staphylococcus aureus* biofilm formation, increase its biofilm dispersion and affect gene expression of important virulence factors of this pathogen. Now we focused on identifying the bioactive molecules, determine their impact on *S. aureus* ability to adhere and invade host epithelial cells, as well as determining the impact of these molecules on host cells. Our results showed that growth of *S. aureus* in the presence of *S. epidermidis* CFCM significantly reduced both adhesion and invasion of *S. aureus* in A549 cells. Cell viability and toxicity were determined using LDH and MTT assays and showed that *S. epidermidis* CFCM at either 5% or 1% concentrations did not significantly impact A549 cells. Biofilm assays demonstrated that *S. epidermidis* CFCM at both concentrations significantly reduce biofilm formation of *S. aureus*. Furthermore, we have successfully obtained purified active fractions of *S. epidermidis* CFCM through High Performance Liquid Chromatography (HPLC) and analyzed their composition by liquid chromatography-mass spectrometry (LC-MS). Our findings highlight the potential of *S. epidermidis*-derived molecules in combating *S. aureus* infections and underscore the importance of further exploring the skin microbiota for therapeutic applications.

#19 – The Computational Design of Peptides Derived from E-cadherin to Modulate the Blood Brain Barrier

Jinyan He[1], Krzysztof Kuczera[1,2], and Teruna J. Siahaan[3]

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

The blood-brain barrier (BBB) can protect the brain from pathogen infections from blood to brain, but at the same time it prevents drugs from entering the brain to treat brain diseases. Previously, two classes of peptides, ADT- and HAV-derived, have been identified, capable of modulating the permeability of the BBB both *in vitro* and *in vivo*. ADT and HAV peptides were derived from the first domains of E-cadherin (EC1), one of many Velcro-like molecules at the cell junctions to seal intercellular space. The goal of our project is to find new and more potent peptides which are also derived from E-cadherin protein, to better modulate the permeability of the BBB. By exploring all possible interactions between EC1–EC1, EC2–EC2 and EC1–EC2 domains using rigid docking, a variety of potential binding poses have been identified, yielding candidate sequences for binding peptides. The proposed peptide candidates were then redocked to E-cadherin and the binding affinity for generated docking poses were computationally evaluated to identify candidates with strong binding affinity. To fully understand the sampled conformations of the peptides and their interconversions, we have also performed molecular dynamics simulations in aqueous solution. From the trajectories, we have constructed kinetic network models to study peptide dynamics and structures, providing microscopic insights into the behavior of these flexible molecules which is difficult to study experimentally. In the next project phase, the

candidate peptide sequences will be computationally optimized for the strongest affinity to E-cadherin. Also, the binding affinity of proposed peptides in cyclic version to E-cadherin protein will also be computationally studied. One peptide candidate was picked and its high affinity for E-cadherin experimentally verified by surface plasmon resonance to validate computational predictions.

#20 – Estrogen Depletion Effects on Lipid Homeostasis and Myelination

Esther Holt[1], Hiroko Kobayashi[2], Dr. Meredith Hartley[1]

[1]Department of Chemistry, The University of Kansas, Lawrence, KS, USA; [2]Department of Neurochemistry, University of Buffalo, Buffalo, NY, USA

Nearly 75% of multiple sclerosis patients are female and have symptoms that improve during pregnancy and worsen during post-menopause, which correlates with changes in estrogen levels within the body.1,2 Studies have shown that the beneficial effects of estrogen on myelination may be attributed to lipid changes in the CNS.1,3 Menopause is associated with worsening symptoms in MS and a decrease in glucose metabolism in the brain which leads to the breakdown of myelin lipids.3 However, the effect of menopause on demyelination and the regulation of myelin lipid pathways has yet to be fully investigated. Our objective is to determine how estrogen loss affects CNS lipid metabolism during demyelination and remyelination. To achieve this goal, we are using the iCKO-*Myrf* (myelin regulatory factor) mouse strain as a model of demyelination, in which tamoxifen is used to induce ablation of *Myrf* and initiate demyelination. To mimic menopause, we perform ovariectomies to deplete estrogen prior to inducing demyelination. The experimental groups include ovariectomized females, sham surgery females, control females and control males. Rotarod and horizontal ladder tests are used to monitor motor disability and recovery of mice. Our preliminary results suggest that estrogen depletion accelerates the onset of motor disability after demyelination. Brain and spinal cord tissue will be collected from experimental mice at weeks 12 and 24 post-tamoxifen injection, which represents peak demyelination and remyelination recovery. Myelin and cellular markers of myelin will be analyzed by BlackGold staining, electron microscopy, and immunofluorescence. Lipidomic analysis will be performed to measure how both phospholipids and sterols are altered with estrogen depletion in demyelination. These studies will define how estrogen loss in the iCKO- *Myrf* mouse model affects remyelination and CNS lipid metabolism during demyelination. These experiments will enable us to identify mechanisms that mediate the connection between menopause and multiple sclerosis.

References:

1. Rankin, K.; Mei, F.; Kim, K.; Shen, Y.; Mayoral, S.; Desponts, C.; Lorrain, D.; Green, A.; Baranzini, S.; Chan, J.; Bove, R., *J. Neurosci. Res.*, **2019**, *39* (12), 2184.
2. Bove, R.; Okai, A.; Houtchens, M.; Elias-Hamp, B.; Lugaresi, A.; Hellwig, K.; Kubala, Havrdová, E., *Front. Neurol.*, **2021**, *12*, 554375.
3. 3. Voskuhl, R.; Itoh, N.; Tassoni, A.; Matsukawa, M.; Ren, E.; Tse, V.; Jang, E.; Suen, T.; Itoh, Y., *PNAS*, **2019**, *116* (20), 10130.

#21 – 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

The University of Kansas Computational Chemical Biology Core (CCB) provides the computational resources and expertise to enhance the productivity of researchers studying infectious diseases. The CCB is able to provide or assist with virtual screening, protein-small molecule docking, binding site prediction, protein modeling and design, prediction of protein stability changes upon mutation, fragment-based probe design, as well as preparation of presentation graphics. The core utilizes the KU Community Cluster at the Advanced Computing Facility for its high-performance computing needs. The KU Community Cluster offers 458 compute nodes with a total of 8,568 compute cores, including 17 nodes that offer GPU-accelerated computing. The CCB specializes in initial hit identification of non-traditional drug targets such as protein-protein or protein-RNA interfaces by offering high-throughput virtual screening via pocket optimization with exemplar screening at protein-protein interfaces and hotspot pharmacophore mimicry of protein-RNA interactions.

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

#22 – Gut Commensal Metabolites Modulate *V. cholerae* Biofilm Growth

Kate Kirby[1], Heidi Pauer[1], Alyssa Pold[1,2], Caetano Antunes[1]

[1]*Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA;* [2]*Department of Biology, University of North Georgia, Dahlonega, GA, 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. Previously, our lab identified *Enterocloster citroniae* as a gut microbiota member that produces metabolites with a dampening effect on *V. cholerae* motility while increasing biofilm formation. This reflects an expected inverse relationship between motility and biofilm formation in bacterial behavior. Our goal is to isolate additional gut microbiota members that show biological activity against *V. cholerae*. After isolating gut microbes from fecal samples, we used ethyl acetate to extract their metabolites and performed a biofilm assay to demonstrate the effects of gut commensal extracts on *V. cholerae* biofilm formation. Of the 87 isolated gut microbiota strains obtained, 28 have been tested so far. Of these 28 samples, 6 showed a significant increase in *V. cholerae* biofilm formation, 4 showed a significant decrease, and 14 showed no significant change. 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 have yet to be identified, including the biofilm-inhibiting strains. We are continuing to isolate, test, and identify more gut bacteria to investigate their effects on *V. cholerae* biofilm growth and motility.

#23 – Using ^{19}F NMR to Study the Membrane Interactions In the N-Terminal Domain of IpaB

Jacob Kroh and Roberto De Guzman

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

The Type III Secretion System (T3SS) is a complex nano-injector employed by infectious gram-negative bacteria, such as *Shigella*, *Salmonella*, *Burkholderia*, and *Yersinia* pestis. This system provides bacterial effector proteins a tunnel from the bacterial cytosol into the host cell through a pore it creates in the membrane. The T3SS is comprised of four highly conserved structures: the base, the needle, the tip complex, and the translocon. Though the individual component proteins may be structurally variable between species, the overall macrostructure and function are homologous. The atomic structure of the translocon is largely unknown. It is a heterooligomeric structure composed of a major and a minor

translocase. In Shigella, these proteins are IpaB and IpaC, respectively. IpaB has a small ectodomain in its N-terminus that is ordered enough for a crystal structure to exist. Using this ectodomain, as well as a slightly larger truncation of the IpaB, our lab wanted to probe the potential for membrane interaction of this domain using ^{19}F NMR. We found that there are indeed portions of this domain that interact with the membrane, as well as portions that do not. We then used these insights to create an interaction map of, and new working model for the membrane interaction of the N-terminal domain of IpaB. This information provides a novel glimpse into a potential mechanism for the insertion of IpaB into the membrane as well as better refinement of its topology around the host membrane-T3SS interface.

#24 – Observed Decrease in Dopamine Release in Zebrafish with Src-I1 Perfusion

Ethan V. Le[1,2], Sayuri Niyangoda[1,2], Michael A. Johnson[1,2]

[1]Department of Chemistry, The University of Kansas, Lawrence, KS, USA; [2]R.N. Adams Institute for Bioanalytical Chemistry, The University of Kansas, Lawrence, KS, USA

Src kinases are a family of tyrosine kinases implicated in the regulation of multiple signaling processes involved in cellular proliferation and differentiation. Furthermore, the presence of the Src tyrosine kinase in the central nervous system suggests its involvement in glutamate release and neural excitation. However, the downstream effect of Src-mediated glutamate regulation on the release of further excitatory neurotransmitters remains unclear. Here we studied the effect of perfusing Src-I1, a Src family tyrosine kinase inhibitor, on dopamine release in zebrafish models using fast-scanning cyclic voltammetry on bare carbon fiber microelectrodes, due to the high similarity between central nervous systems of zebrafish and humans. We found that perfusing Src-I1 appeared to lead to a statistically significant decrease in dopamine release, suggesting that the inhibition of glutamate release led to decreased activation of the NMDA receptor, resulting in the observed decrease in dopamine release. This finding shows promise as a way to modulate the release of neurotransmitters in zebrafish models using Src kinase inhibitors to investigate neurological disease.

#25 – Exploration and Analysis of the Effects of Hydrogen Bonding on the Basicity of Mn^{III}-hydroxo Complexes in Proton-Coupled Electron Transfer (PCET) Reactions

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

[1]Department of Chemistry and Center for Environmentally Beneficial Catalysis, The University of Kansas, Lawrence, KS, USA; [2]J. Heyrovský Institute of Physical Chemistry, The Czech Academy of Sciences, Dolejškova 3, Prague 8, Czech Republic

Mn^{III}-hydroxo complexes are key intermediates in the metalloenzymes manganese lipoxygenase and manganese superoxide dismutase, where proton-coupled electron transfer reactions occur using a $Mn^{III}OH/Mn^{II}OH_2$ couple. In the active sites of these enzymes, there are amino acids that are capable of hydrogen bonding with the hydroxo ligand. Recently, we used two synthetic Mn^{III}-hydroxo model complexes to reveal that hydrogen bonding influences the rate of reactions between the Mn^{III}-hydroxo complexes and phenolic substrates in PCET reactions. In this work, we assess the role of hydrogen bonding as it relates to the basicity of the hydroxo ligand and explore how this changes PCET reactivity. Using experimental and computational methods, we were able to gain information about the thermodynamic properties of the reactions with different substrates. For the more acidic substrates, the rates fall outside of the trend, which we propose corresponds to a change in mechanism.

#26 – Strategies for Synthesis 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

Macrocycles play important roles in drug discovery for the development of novel pharmaceuticals, in synthetic chemistry as metal-binding ligands, and in agriculture as pesticides. Despite a wealth of reports of macrocycles in the literature, sulfur-containing macrocycles are relatively underpopulated in the literature. Our interest in the development of novel sulfur-containing heterocycles has led us to develop a strategy for facile synthesis of 14-membered sulfamide-containing macrocycles through a 3-component coupling involving amino and alcohol components possessing olefins/acetylenes with chlorosulfonyl isocyanate. Ring-closing metathesis and ring-closing enyne metathesis have been utilized for cyclization. Following synthesis of this scaffold, three differentially protected nitrogen atoms within the 14-membered core allow for selective deprotection and installation of functional groups. This synthetic strategy has potential for the generation of many analogs, as the components used for coupling can be swapped out to generate a variety of scaffolds.

#27 – HSV-1 ICP0 Dimer Mutants Impair Protein Functions and Viral Replication

Erick McCloskey[1], Maithri Kashipathy[2], Anne Cooper[3], Philip Gao[3], David K. Johnson[4], Kevin P. Battaile[5], Scott Lovell[2], and David J. Davido[1]

[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; [2]Protein Structure and X-Ray Crystallography Laboratory, University of Kansas, Lawrence, KS, USA; [3]Protein Production Group, University of Kansas, Lawrence, KS, USA; [4]Computational Chemical Biology Core, University of Kansas, Lawrence, KS, USA; [5]NYX, New York Structural Biology Center, Upton, New York, USA

Infected cell protein 0 (ICP0) is an immediate-early regulatory protein of herpes simplex virus 1 (HSV-1) that possesses E3 ubiquitin ligase activity. ICP0 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 relates to these functions. ICP0 was purified and analyzed by X-ray crystallography, revealing a composition of nine β-sheets and two α-helices. Two neighboring β-sheets from each monomer "reach" into the adjacent subunit during dimer formation, generating two β-barrel-like motifs. Structural protein database searches indicate the fold/structure is novel, and the dimer is held together by an extensive network of hydrogen bonds. This structure allowed for the identification of residues involved in dimer formation. These residues were substituted to alanine to prevent dimerization, with the goal of preserving other functional domains in ICP0's C-terminus. Preliminary data from promoter activation assays suggest ICP0 dimer mutants are unable to effectively stimulate an HSV-1 promoter to the same degree as wild-type (WT) ICP0, and are unable to fully complement the replication of an ICP0-null mutant. Interestingly, these mutant forms of ICP0 show increased stability during infection compared to WT ICP0. Lastly, an 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. Future studies will determine how ICP0 dimerization regulates its functions and the HSV-1 life cycle.

#28 – 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, The University of Kansas, Lawrence, KS, USA; [2]Department of Molecular Biosciences, The 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 a BD FACSymphony™ S6 Cell Sorter, a BD FACSAria™ Fusion cell sorter, a Cytek™ Aurora spectral flow cytometer, an Agilent NovoCyte Advanteon conventional flow cytometer, and other supplemental assay instrumentation. 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 manages a FlowJo™ site license for data analysis software, and 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

#29 – FusA1 Mutations Increase Ceftazidime Resistance through Quorum Sensing and Stringent Response in *Pseudomonas aeruginosa*

Brielle M. McKee[1]; Rhea Abisado-Duque[2]; Kade Townsend[1]; Kate Woods[1]; Luke Woodward[1]; Josephine R. Chandler[1]

[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; [2]Department of Biology, Ateneo de Manila University, Quezon City, PHL

Pseudomonas aeruginosa is a common multi-drug resistant human pathogen that can cause long-term and difficult to treat infections. During *P. aeruginosa* infections, adaptive mutations commonly emerge and alter resistance to clinically relevant antibiotics, such as tobramycin and ceftazidime. One of the most common mutations is in *fusA1*, which encodes a translation accessory protein. Our prior work suggested that *fusA1* mutations may modulate resistance to ceftazidime, although little is known about this potential functional link. We tested the MIC of several FusA1 mutants and discovered that they yield a large range of effects on resistance, with one decreasing resistance and most others increasing resistance, including FusA1 L40Q, which increased resistance ~5-fold compared to the PA14 wildtype. Resistance of this mutant was highly dependent on quorum sensing and stringent response, which are both gene regulation networks. Although ceftazidime's primary mechanism of action is cell-wall synthesis inhibition, the drug's lethality is known to be enhanced by the generation of reactive oxygen species (ROS). We hypothesized that quorum sensing and stringent response protect against ceftazidime-induced ROS by activating ROS-detoxifying enzymes, such as KatA. By synthetically inducing KatA in the *lasR* mutant, we observed restored resistance, supporting the idea that LasR protects against ROS-dependent ceftazidime lethality. However, no effect to resistance was observed when KatA was

expressed in the stringent response mutant, indicating that quorum sensing and stringent response are regulating resistance of ceftazidime through different mechanisms. We are currently working to understand the regulatory effects of FusA1 mutants on stringent response and quorum sensing. Overall, our results provide new insight into ceftazidime resistance mechanisms in *P. aeruginosa* that could have implications for the development of novel anti-infectives.

#30 – The Three-component Signal Transduction System YesLMN of *Enterococcus faecalis* Senses Host Glycans to Activate Expression of an ABC Transporter Required for Host Glycan Import

Abdulrahman M. Naeem, Janie B. Rainer, Tolulope I. Ade, Zakria H. Abdullahi, and Lynn E. Hancock

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

Enterococcus faecalis, an opportunistic pathogen that normally inhabits the gut in humans has the capacity to utilize a wide range of carbohydrate sources. In *E. faecalis*, σ^{54} (RpoN) controls the expression of multiple phosphotransferase systems (PTS) responsible for metabolism of carbon. Through previous work in our lab, it was shown that in the absence of RpoN, alternative carbon sources are required to maintain bacterial growth when grown on glucose as the principal carbon source. Prior transcriptional analysis comparing parental strain V583 with its isogenic rpoN deletion identified the most differentially expressed genes in the *rpoN* mutant comprising an operon that includes a predicted ABC transporter, EF2223-21 and a three-component signal transduction system (YesLMN). Since YesN is a predicted response regulator, we constructed a *yesN* mutant and assessed its contribution to the regulation of the operon, as well as potentially other genes regulated by YesN by RNA-seq analysis and confirmed the transcriptomic data by qRT-PCR and luciferase promoter fusions. To assess the contribution of YesL and YesM, a predicted ancillary membrane protein and a membrane bound sensor histidine kinase, we constructed in-frame deletion mutants of both genes and complemented those defects by use of an ectopic integration system to address potential polar effects on YesN regulation and activity. A luciferase reporter transcriptionally fused to *ef2223* (the first gene in the operon) allowed us to also address the host glycans that are sensed in a YesLMN-dependent manner and data show that high-mannose type N-linked glycans are sensed by YesLMN.

#31 – Characterization of anti-CA125 Monoclonal Antibodies by Western Blot and ELISA

Trisha Nair, Caitlin R. McEntee, Chien-Wei Wang, Rebecca J. Whelan

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

To improve ovarian cancer survival rates, better diagnostic tools must be developed to detect the cancer at earlier stages. The current FDA-approved ovarian cancer surveillance test, the CA125 blood test, monitors levels of CA125 in the blood using a sandwich ELISA (enzyme-linked immunosorbent assay) with the M11 and OC125 antibodies. CA125 is an epitope on MUC16, a heavily glycosylated protein biomarker containing a region of 19 tandem repeats. CA125 is located within the tandem repeat region, but little is known about its exact location, structure, and role in ovarian cancer progression. To gain insight into CA125's structure, we probed 16 of the 19 individual repeats with M11-like and OC125-like monoclonal antibodies, which have been reported to bind to CA125 similarly to the clinically used antibodies. This project aimed to characterize two M11-like clones (M002203 and M77161) and two OC125-like clones (M002201 and X306) using Western blot and indirect ELISA techniques. Western blot was used to detect binding between the antibody and denatured repeat proteins, revealing potential linear epitopes. ELISA was used to detect binding between the antibody and proteins in their native state, examining both linear and conformational epitopes. The two M11-like clones displayed binding to

more repeats across the tandem repeat region compared to the two OC125-like clones, which bound to less repeats across this region. The M11-like clones also exhibited different binding patterns while the OC125-like clones showed similar binding patterns on the Western blot and ELISA. Future work will utilize surface plasmon resonance (SPR) as an additional method for characterization of these antibodies. In addition, more antibody-antigen binding interactions may be studied to gain further insight into CA125's location and structure.

#32 – Bioactive Small Molecules Produced by the Gut Commensal *Enterocloster citroniae* Modulate *Vibrio cholerae* Behavior

Saeideh Nasiri, and Caetano Antunes

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

Microorganisms living in the human gut have long been known to protect us against pathogenic bacteria. Our group has previously shown that microbiome-derived small molecules elicit strong transcriptional responses in multiple enteric pathogens. Often, genes differentially regulated in response to bioactive compounds encode properties related to virulence and host interactions. Here, we focus on one gut microbiota species, *Enterocloster citroniae,* and demonstrate that extracts from pure cultures of this commensal induce biofilm formation and dampen motility in *Vibrio cholerae.* We also established infection protocols using *V. cholerae* and a colorectal epithelial cell line (HT-29) to investigate the impact of *E. citroniae* extracts on *V. cholerae* interactions with host cells. Our data showed that *E. citroniae* inhibits *V. cholerae* adhesion to HT-29 cells. Also, *E. citroniae* extracts were tested in cytotoxicity and viability assays and our data showed that the compounds produced by this commensal are not cytotoxic. Furthermore, to determine the chemical nature of active compounds, a series of purification methods using reversed-phased High Performance Liquid Chromatography (HPLC) were employed. Unfortunately, however, no bioactive fractions were obtained. Therefore, we are currently focusing our efforts on identifying chromatographic conditions that allow us to purify the active compounds, including the use of normal-phase HPLC. Mass spectrometry and nuclear magnetic resonance (NMR) assays will be performed on active fractions to determine the chemical composition of active compounds. To that end, we used ^{1}H and ^{13}C NMR to profile the chemical composition of extracts from active strains, and common features could be detected. Once compounds are purified and identified, we will investigate their effects on *V. cholerae* behavior. This work will shed light on *V. cholerae* interactions with its host and the associated microbiota through the lens of chemical biology.

#33 – Characterizing *C. acnes* and *Staphylococcus* spp. in Polymicrobial Biofilms

Bren Den Ng, Danielle S. Williams, Rosana B. R. Ferreira

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

Polymicrobial biofilms are frequent causes of chronic infections and a serious medical problem due to antimicrobial resistance. *Staphylococcus* spp. and *Cutibacterium acnes* are common skin commensals and are usually beneficial, but can cause chronic infections of prosthetic- devices and are frequently co-isolated from these infections. Our group has shown that *S. hominis* and *S. lugdunensis* produce substantially more biofilm when co-cultured with *C. acnes* compared to monocultures. This phenotype is specific to this combination of species. In this project, we aim to better characterize the interactions among these three species, focusing on identifying the mechanism by which the interaction with *C. acnes* causes increased biofilm formation in *S. lugdunensis* and *S. hominis*. For that, we inoculated *S. lugdunensis* and *C. acnes* in monoculture or in co-culture using different concentrations of each species and incubated them at 37 ^{o}C for 72h under anaerobic conditions and assay for biofilm production. We found that concentrations as low as 1% of *S. lugdunensis* can still display the same strong biofilm

formation in the presence of *C. acnes.* However, at least 10% of *C. acnes* is needed for the phenotype to be observed in the presence of *S. lugdunensis*, suggesting a major role for *C. acnes* on this interaction. We now plan to quantify each species in polymicrobial biofilms over time and evaluate the impact of co-culture on global gene expression of each species. The results and subsequent characterization of the interaction will allow us to combat the polymicrobial infection by targeting the mechanisms involved.

#34 – Microbiome-Derived Small Molecules Promote Growth of a *Vibrio cholerae* MbaA Mutant with Reduced Cyclic di-GMP Synthesis

Vivian Nguyen, Saeideh Nasiri, Caetano Antunes

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

Vibrio cholerae, the causative agent of cholera, is a water and foodborne enteric pathogen. During intestinal colonization, *V. cholerae* interacts with the human gut microbiota, a rich community of microorganisms. This microbiota is metabolically active, and constantly produces and releases chemical compounds into the gut environment. Our group has shown that these compounds can be bioactive, affecting gene expression of multiple enteric pathogens. In *V. cholerae*, we have shown that microbiota-derived compounds have a drastic impact on global gene expression, with genes involved in host colonization being modulated. Our lab has previously revealed that a specific gut commensal, *Enterocloster citroniae,* produces compounds that can alter *V. cholerae* gene expression and behavior. Specifically, extracts of *E. citroniae* culture supernatants were shown to inhibit swimming motility and induce biofilm formation in *V. cholerae.* One of the *V. cholerae* proteins that are known to be involved in regulating biofilm formation through the modulation of cellular c-di-GMP levels is MbaA. Therefore, we hypothesized that the effect of *E. citroniae* extracts on *V. cholerae* biofilm formation could occur through MbaA. Here, we used an *mbaA* point mutant (MbaA*) deficient in c-di-GMP synthesis to investigate its response to *E. citroniae* extracts. Interestingly, we found that the growth rate of the MbaA* strain is increased compared to the wild-type strain, but only when the strains are grown in the presence of *E. citroniae* extracts. We also showed that in the MbaA* *V. cholerae* strain, biofilm formation was induced significantly more than in the wild-type strain by organic extracts of the culture medium, Brain Heart Infusion, as well as *E. citroniae* culture supernatants. Interestingly, however, swimming motility assays using the MbaA* strain showed the same pattern of motility inhibition observed with the wild-type strain. Future studies will focus on determining the mechanism of growth promotion observed in the MbaA* strain during growth in the presence of *E. citroniae* extracts.

#35 – Mutations Affecting Different Biochemical Functions of the Coronavirus Macrodomain, Mac1, Indicate that it Promotes Multiple Stages of the Viral Replication Cycle.

Joseph J. O'Connor[1], Anu Roy[2], Yousef M. Alhammad[1], Reem Khattabi[1], Philip Gao[3], and Anthony R. Fehr[1]

[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; [2]High Throughput Screening Laboratory, University of Kansas, Lawrence, KS, USA; [3]Protein Production Group, University of Kansas, Lawrence, KS, USA

All coronaviruses (CoVs) encode a conserved macrodomain, termed Mac1, in non-structural protein 3 (nsp3) which binds and hydrolyzes ADP-ribose covalently attached to proteins. Mac1 is a key virulence factor that counters antiviral ADP-ribosylatransferase (PARP) activity. Previously, we found that MHV with a mutation in the adenine binding site, MHV-D1329A, was extremely attenuated in all cell culture models of infection, as opposed to MHV-N1347A, which only has a replication defect in bone-marrow derived macrophages (BMDMs). Interestingly, an N1347A/D1329A double mutant was unrecoverable, indicating an essential role for Mac1 in infection. Based on this prior work, we hypothesized that these

mutations may impact different stages of the viral lifecycle. First, to clarify the impact these mutations have on the biochemical activities of Mac1, we generated recombinant SARS-CoV-2 Mac1 proteins encoding the same mutations. As expected, the D-A mutation was extremely defective in ADP-ribose binding, but maintained some enzyme activity, while the N-A mutation had nearly WT levels of ADP-ribose binding but had low enzyme activity, confirming that these mutations have different effects on the biochemical functions of Mac1. Next, we analyzed how MHV D1329A and N1347A viruses impact the viral lifecycle. In BMDMs, N1347A infected produced normal levels of viral genomic and sub-genomic RNA, but had reduced levels of viral protein, indicating a defect in protein translation. In contrast D1329A produced viral non-structural protein 3 early during infection, but displayed a large defect in the accumulation of viral genomic and sub-genomic RNA compared to WT or N1347A. These results suggest that Mac1 binding and enzymatic activities are critical for different stages of the viral lifecycle, demonstrating the critical importance of Mac1 for MHV replication.

#36 – Synaptic Degeneration and Reduced Lifespan in Familial Alzheimer's Disease *C. elegans* Model Not Due to Simple Loss of Function of γ-secretase

Caitlin Overmeyer[1], Brian D. Ackley[2], and Michael S. Wolfe[3]

[1]Department of Pharmocology & 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*

Early-onset Familial Alzheimer's Disease (FAD) is caused by autosomal dominant mutations in Presenilin 1, Presenilin 2, and Amyloid Precursor Protein (APP). Presenilin is the known catalytic component of the γ-secretase enzyme complex, which cleaves APP. APP cleavage produces amyloid-β which has been thought to lead to the neurodegeneration seen in Alzheimer's Disease. However, targeted treatments for amyloid-β and γ-secretase inhibition have been largely ineffective with severe side effects. Previous biochemical work from our lab demonstrates that FAD mutations in Presenilin lead to impaired cleavage by gamma-secretase. Using transgenic *Caenorhabditis elegans* containing human Presenilin 1 as a model organism in which to study FAD mutations, we have shown that these mutations lead to synaptic degeneration and reduce lifespan. Interestingly, an FAD mutation in Presenilin does not need the presence of wild-type human C99 to reduce lifespan, and is amyloid-β independent. These observations led us to the novel hypothesis that FAD mutations cause the enzyme-substrate complex to stall, leading to the phenotype. In this study, we wanted to investigate whether or not the phenotype is due to a loss of function in the enzyme, or to new gain of toxic function due to the stalled complex. Utilizing the D257A Presenilin mutation that leads to a catalytically dead enzyme that cannot interact with substrate, we scored animals for both synaptic degeneration and lifespan. When compared to parental strain *juIs1*, neither phenotype was seen, indicating the effect is not due to a simple loss of function of the enzyme. This observation supports the stalled-complex theory, that interaction with substrate is necessary to form the enzyme-substrate complex that leads to synaptic degeneration and reduces lifespan.

#37 – Targeting Coronavirus Mac1: Identification of a Drug-Resistant Mutant Confirms Inhibitor Specificity

Jessica J. Pfannenstiel[1], Michelle D. Woolridge [2], Dana Ferraris[3], Anthony R. Fehr[1]

[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS, USA; [2]Department of Biological Sciences, University of Missouri, Columbia, MO, USA; [3]McDaniel College Department of Chemistry, McDaniel College, Westminster, MD, USA

Coronaviruses (CoVs) cause significant diseases in humans and animals, with recent outbreaks of severe human disease caused by SARS-CoV, MERS-CoV, and, more recently, SARS-CoV-2. All CoVs encode for a macrodomain protein (Mac1), which removes ADP-ribose from target proteins, a post-translational modification linked to virus suppression and interferon (IFN) induction. In murine hepatitis virus (MHV), a model coronavirus, Mac1 is essential for replication in cell culture. For SARS-CoV-2, a complete deletion Mac1 is viable but exhibits a significant replication defect when challenged with IFN-γ and is highly attenuated in mice. These data illustrate the ability of Mac1 to counter host responses and suggest that it is a potential therapeutic target for CoVs. To explore therapeutic options, we first developed several pyrrolo-pyrimidine derivatives that inhibited Mac1 *in vitro* but did not reduce CoV replication, likely due to poor cell permeability. We then developed more hydrophobic derivatives and identified **5c** and **6e**, which inhibited Mac1 activity *in vitro* and repressed MHV and SARS-CoV-2 replication. MHV was passaged with **5c** to identify drug-resistant mutations and confirm specificity. We sequenced drug-resistant isolates of MHV and identified a G73E mutation in Mac1. Engineering this mutation into MHV confirmed that it conferred resistance to **5c**, as the mutant virus showed enhanced replication compared to wild-type under **5c** treatment. However, the G73E virus exhibited reduced replication compared to WT without **5c**, suggesting a fitness tradeoff. Further viral genome sequencing will be performed to identify additional mutations and determine mechanisms associated with Mac1 drug resistance.

#38 – The Impact of *PTPN22* on the B Cell Landscape During Viral Infection

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

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

Persistent viral infections affect millions of people globally. B cells play crucial roles in viral clearance and orchestrating immune memory during persistent infections. They assist CD4+ T cells as antigen-presenting cells and are the exclusive producers of antibodies. Protein Tyrosine Phosphatase Non-Receptor Type 22 (*PTPN22*), is expressed in all immune cells, encoding for PEP (mice)/LYP (humans). Further, mice lacking *Ptpn22* (PEP-Null) can clear persistent Lymphocytic choriomeningitis virus clone 13 (LCMV-cl13) infection, whereas PEP-WT mice cannot. However, the role of *Ptpn22* in B cells during virus infection is unknown. **We hypothesize that the lack of *Ptpn22* establishes a selection bias promoting Follicular over Marginal Zone B cells, changing the overall B cell landscape during viral infection.** To assess this, we characterized B cell subsets in PEP-WT and PEP-Null mice at a naïve state and during LCMV-cl13 infection. In naïve animals, PEP-Null mice have more Follicular and Plasmablast B cells compared to PEP-WT mice. Following LCMV-cl13 infection, PEP-Null mice have less Follicular B cells, but more Plasmablasts compared to PEP-WT. This suggests that *Ptpn22* could be impacting fast-acting Plasmablast antibody production. In addition, PEP-Null mice are producing more antibodies at an earlier time point than PEP-WT mice. Therefore, clearance of LCMV-cl13 in PEP-Null mice could be, in part, due to these fast-acting antibodies. Defining these mechanisms would advance our understanding of the critical role B cells play in persistent infections and could lead to potential avenues for vaccine development.

#39 – Ferric 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. Metal-hydroxo complexes like lipoxygenases (LOXs) use Fe^{III}-OH intermediates to oxidize fatty acids. Out of the few examples of Fe^{III}-OH bioinspired models, the reactivity of these complexes in C-H bond oxidation varies significantly. While some Fe^{III}-OH complexes can attack C-H bonds, most synthetic Fe^{III}-OH complexes exhibit low reactivity. The variation in reactivity seems to depend on the ligand environment, though the specific features responsible remain unclear. This study explores the synthesis and reactivity of two Fe^{III}-OH complexes. The first Fe^{III}-OH complex is generated via the interconversion of an Fe^{II}-H_2O species, and its proton-coupled electron transfer (CPET) reactivity is examined using TEMPOH as a substrate. In efforts to isolate a mononuclear Fe^{III}-OH for the second complex, an oxo-bridged binuclear Fe^{III} species was obtained. The acid-mediated conversion of this oxo-bridged dimer into the mononuclear Fe^{III}-OH species is also investigated. The newly synthesized Fe^{III}-OH complexes will help assess how ligand-sphere changes affect thermodynamic characteristics and CPET reactivity.

#40 – Synthesis of multiantigen Fc Bifunctional Peptide Inhibitors as Immune Modulators for EAE Mice

Marcela Solis Rodriguez, Teruna J Siahaan, and Thomas Tolbert

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

Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system (CNS), which is triggered by the self-recognition of the myelin sheath proteins like proteolipid protein (PLP), myelin basic protein (MBP), and myelin oligodendrocyte glycoprotein (MOG), the three major antigens for this disease. Clinically, MS is classified in 4 types; relapsing-remitting MS (RRMS), primary-progressive MS (PPMS), secondary-progressive MS (SPMS), and progressive-relapsing MS (PRMS). Being RRMS the least severe of all, and commonly the first stage for 85% of the patients with MS, otherwise PRMS is the most severe one with higher mortality rates. It is worth mentioning that in the stages of MS, the proinflammatory T cells have shown different preference for the myelin sheath proteins. Indeed, at the onset of the disease or in RRMS the major antigens are PLP and/or MBP; however, in SPMS and PRMS the three antigens are the main targets.

The treatment available for MS is limited to 20 FDA approved products from which just two can be used for progressive stages of the disease. In addition, their action mechanisms lead to general immunosuppression causing opportunistic diseases in the patients.

This project aims to the development of immuno-specific treatments for the relapsing and progressive versions of MS modulating the proinflammatory T cells that recognize the principal antigens involved in MS. This through the bifunctional peptide inhibitors (BPIs) that consist of three components; the first is any of the myelin sheath antigenic peptides, a linker (IgG1 Fc), and the signal-2 blocking peptide like $CD11a_{237-246}$-derived peptide (LABL). The conjugation of both peptides leads to the inhibition of costimulatory signals that differentiates the lymphocytes as pro-inflammatory for the specific antigenic peptide. The efficacy of BPIs has been evaluated in experimental autoimmune encephalomyelitis (EAE) mouse models, showing promising results lowering the symptoms in the EAE models. However, following the antigenic spreading event (as the disease progresses, the most antigens are recognized), the next step is to develop a multiantigen BPI that contains the three antigenic peptides. To approach it, we consider the lysine residues on the Fc (linker) to conjugate the antigenic peptides through the NHS-ester maleimide chemistry. To develop this system, we decided to conjugate every antigenic peptide separately due to their hydrophobicity, evaluate the multi-conjugation behavior, and then their activity on the EAE model.

#41 – Inhibitors of CDK-1 and CDK-2 Diminish HSV-1 Immediate-Early Gene Expression

Maxim Rodzkin, Drew Honeycutt, and David Davido

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

Herpes simplex virus 1 (HSV-1) is a DNA-enveloped virus causing oral, facial, or genital sores and, in severe cases, keratitis and encephalitis. As an obligate intracellular pathogen, HSV-1 exploits cellular factors, including host transcription machinery. Cyclin-dependent kinases (CDKs) are crucial regulators of transcription, interacting with components of the transcription machinery and modifying chromatin structure. Evidence suggests that cell cycle-related CDKs, such as CDK-1 and CDK-2, may be involved in both host and viral gene transcription. Previous studies from our lab have shown that the broad-spectrum CDK inhibitor Roscovitine reduces HSV-1 immediate-early (IE) transcript levels. To determine which specific CDKs modulate HSV-1 replication and gene expression, we performed viral yield assays using inhibitors of CDK-1, CDK-2, and CDKs-4/6, observing that inhibitors of CDK-1 and CDK-2 significantly impaired wild-type virus replication. Subsequent reverse transcription-qPCR and western blot experiments showed that CDK-1 or CDK-2 inhibition led to a substantial reduction in IE gene expression as early as 3 hours post-infection (hpi). To identify CDK-1 targets involved in IE transcription, we performed high-throughput tandem mass tag (TMT) mass spectrometry to examine changes in the host and viral phosphoproteomes early during infection upon CDK-1 inhibition. These phosphoproteomic analyses suggest that the large subunit of RNA polymerase II is one of several host factors potentially phosphorylated by CDK-1. We propose that CDK-1 phosphorylates RNA polymerase II to stimulate viral IE transcription. Future experiments will examine the roles of CDK-1 and CDK-2 and their effects on specific targets in the regulation of HSV-1 transcription.

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

Anu 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.

#43 – Autoimmunity associated PEP-R619W allele distinctly Regulates Type I Interferon Signaling in Dendritic Cells and CD8 T Cells

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

Type I interferons (IFN-I) enhance dendritic cell (DC) antigen presentation and regulate T cell activity, thus influencing the outcomes of viral infections. The autoimmune-associated PTPN22 allele, PEP-R619W, impacts immune responses but is poorly studied in viral infections. We found that PEP-R619W mice clear Lymphocytic choriomeningitis virus clone 13 (LCMV-clone13) more effectively than wild-type (PEP-WT) mice, coupled with enhanced antiviral immunity. However, the specific signaling mechanisms driving this clearance are unknown. Using CRISPR/Cas9-engineered PEP-R619W mice, we tested the hypothesis that **PEP-R619W alters IFN-I signaling to improve DC and T cell functions**. Post-infection, PEP-R619W DCs show reduced infection, increased CD86, and decreased PD-L1 expression, both in vivo and ex vivo. Infected PEP WT and PEP-R619W DCs display differential gene expression profiles, including higher Jak3 expression in PEP-R619W DCs. Additionally, we show that PEP-R619W enhances IFN-I signaling in DCs. However, PEP-R619W CD8 T cells exhibit less IFN-I signaling. This study highlights that PEP-R619W induces pleiotropic and unique effects across different cell types to prevent immune dysfunction during chronic viral infections.

#44 – Repurposing an Anti-Tumor Drug Candidate for Targeting Bacterial ATP-Dependent Proteases

Zachary Spaulding, Sunitha Shiva, Chathurange B. Ranaweera, Susan Whitaker, and Michal Zolkiewski

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

Protein turnover is a critical process for maintaining proteostasis. The bacterial AAA+ chaperones ClpX and ClpA, coupled with the peptidase ClpP form complexes (ClpXP and ClpAP, respectively) which mediate the degradation of proteins in many bacterial species by threading a substrate through the central pore of the chaperone into the proteolytic site of ClpP. Due to the essential function of these proteins in cell survival, they make for promising targets of novel antimicrobial drugs. Another AAA+ chaperone, ClpB, does not bind to ClpP but bears a similar function of threading substrates through a central channel for subsequent reactivation by other chaperones. We have previously discovered that N2,N4-dibenzylquinazoline-2,4-diamine (DBeQ), a small molecule inhibitor of the mammalian AAA+ protein p97, an anti-tumor target, inhibits ClpB and suppresses the growth of *E. coli*. In this study, we investigated the effects of DBeQ on the engineered ClpB variant, BAP that, like ClpX and ClpA, binds to ClpP and mediates degradation of substrates, instead of their reactivation. We found that DBeQ inhibits the BAP/ClpP-mediated degradation of casein with an apparent IC50 ~ 7μM, but does not affect the intrinsic peptidase activity of ClpP. The loss of BAP/ClpP activity is linked to DBeQ-induced dissociation of the BAP-ClpP complex, as determined by sedimentation velocity, size exclusion chromatography, and dynamic light scattering experiments. Despite the similarities between members of the Clp AAA+ family, DBeQ inhibits ClpA and ClpX with a lower potency than BAP/ClpB. This demonstrates that DBeQ shows a significant degree of selectivity towards BAP/ClpB.

#45 – Validation of a Proteoliposome γ-Secretase Assay to Investigate Cholesterol's Effect on γ-Secretase Function

Tristan Sprague[1] and Michael Wolfe[2]

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

γ-Secretase is a membrane protease that catalyzes the proteolysis of other membrane protein's transmembrane domains. γ-Secretase is best known for its role in forming amyloid β peptide (Aβ) from amyloid precursor protein, a process that ultimately results in the amyloid plaques commonly found in the brains of patients suffering from Alzheimer's disease. γ-Secretase also plays a central part in many other cellular processes, such as Notch signaling. Despite γ-secretase's important roles in cell function, relatively little is known about how the membrane's properties support γ-secretase function. We describe a newly revised proteoliposome-based assay system capable of investigating the relationship between the membrane and γ-secretase. We can incorporate γ-secretase and an APP-based substrate into the liposomes formed by our protocol, such that enzymatic activity is present only in the proteoliposomes after reconstitution. We also find that cholesterol increases γ-secretase activity differently in proteoliposome-based assays versus detergent-based assays and discuss some of the implications. This new method will enable a better description of how γ-secretase interacts with its lipid environment.

#46 – Bioactive Small Molecules from the Human Gut Microbiome Modulate *Salmonella enterica* Invasion Gene Expression

Chukwuma G. Udensi, Caetano Antunes

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

The human gut harbors a vast community of microorganisms known as the gut microbiome, which plays a crucial role in human health by preventing pathogen invasion. Gut commensals achieve this by competing for nutrients, attachment sites, and other unknown mechanisms. Here, we demonstrate that ethyl acetate extracts from pure cultures of *Enterocloster citroniae* and *Enterocloster aldenensis*, human gut commensals, suppress the expression of *hilA*, a key regulator of *Salmonella* host cell invasion. We previously showed that aromatic compounds from the human gut metabolome, including 3,4-dimethylbenzoic acid (DMB), strongly repress *hilA* expression. Here, we screened aromatic compounds from the Infectious Disease Assay Development Core library for compounds structurally related to DMB and identified other small aromatics with anti-invasion activity. Furthermore, to identify the chemical cues produced by *E. citroniae* responsible for the inhibitory activity against *Salmonella* invasion gene expression, bioactive compounds were separated using Reverse Phase High-Performance Liquid Chromatography on a C_{18} column. Surprisingly, no fractions showed activity, suggesting that a compound mixture is required for activity. Nevertheless, we will continue our HPLC efforts using different chromatographic methods. Once active fractions are obtained, mass spectrometry and nuclear magnetic resonance spectroscopy will be performed to identify bioactive compounds. The effect of pure bioactive compounds on *Salmonella* gene expression will be monitored using quantitative real-time PCR targeting invasion and other virulence genes. Together, these results will enhance our understanding of host-pathogen interactions and could lead to the development of novel small-molecule inhibitors against enteric pathogens in humans and animals.

#47 – Comparison of Parent Antigenic Peptides and Bifunctional Peptide Inhibitors to Modulate Autoimmunity

Andrea L. Villela[1,2], Su J. 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

Objective: According to the National Institutes of Health, up to 23.5 million people in the United States suffer from an autoimmune disease and their prevalence is rising.[1] Current therapeutic regimes utilize immunosuppressive agents, thus halting immune cells from recognizing and attacking pathogens. However, lowering systemic immunity can make patients susceptible to other diseases and opportunistic infection by pathogens. Further research is needed to find effective treatments that will regulate a specific subpopulation of the immune system that attacks itself while continuing specificity to fight foreign pathogens. This study aims to evaluate and understand the effect of antigen-specific immunomodulatory peptides on immune activation across various cell lines both *in vitro* and *in vivo*.

Methods: Mouse bone-marrow derived dendritic cells (BMDCs), and splenocytes were harvested from C57BL/6 mice. In BMDC and splenocyte activation assays, cells were plated and treated with various concentrations (10 uM and 1 uM) of five peptides ($OVA_{323-339}$, $OVA_{257-264}$, LABL, $OVA_{323-339}$BPI, $OVA_{257-264}$BPI), and imiquimod (positive control). After 48 h incubation, cells were harvested, and supernatants were collected. The BMDCs were then washed and stained with fluorescently labeled mAbs to measure costimulatory molecule expression (CD40, CD80, CD86) and for splenocytes the cells were stained with fluorescently labeled mAbs (CD25+, Foxp3+, IL-10+) for flow cytometry. Enzyme-linked immunosorbent assays (ELISA) kits were used to examine the expression of IL-10 and TNF-alpha.

Results: When comparing the parent antigenic peptide to bifunctional peptide molecule (BPI), OVA339BPI had a significant increase in IL-10 expression during *in vitro* assays. OVA339BPI significantly increased the frequency of regulatory T cells (Tregs) compared to its parent antigenic peptide and the control group. LDH assay data demonstrated that peptides have negligible cytotoxicity against BMDCs. These peptides did not aggregate in cell media because aggregation could make these peptides inactive. *In vivo*, these peptides increased in IL-10 levels in splenic macrophage as well as upregulation of Foxp3+ and IL-10+ in both CD4+ and CD8+ T cells. Future studies are needed to identify other cell markers and cytokines that are affected upon immune cell exposure by these peptides.

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

#48 – Electrochemiluminescence Reporting for Microchip Electrophoresis with Electrochemical Detection using a Bipolar Electrode: Proof of Concept with Reducible Analytes

Indika K. Warnakula[1,2], Soum Barua[3] and 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 oxygen and nitrogen species (RNOS) are involved in a number of disease states including Alzheimer's disease. Most RNOS and their reaction products are electrochemically active and can be detected using either oxidative or reductive electrochemical detection. However, the majority of applications for the determination of these species have used oxidative detection. The primary reason for this is the high background currents due to the reduction of dissolved oxygen at most negative potentials, leading to higher limits of detection than the oxidative mode. Another strategy is to couple the electrochemical reduction to an optical reporting system; in this case a luminescence-based system. Our group recently reported the development of a microchip electrophoresis system using a bipolar

electrode and fluorescence reporting. Using this system, different polypeptides, including some nitrotyrosine-containing species were detected with this approach. In the current work, the development of a microchip electrophoresis system using reductive electrochemical detection using a bipolar electrode coupled with electrochemiluminescence is described.

The present detection system consists of a PDMS simple-t microchip aligned with a 15 μm PPF electrode (electrophoresis system) and a PDMS straight channel aligned with a 35 μm PPF electrode (reporter channel). Both electrodes were aligned in an in-channel configuration and connected using a copper wire. A voltage was applied across the separation and reporter channels to generate electroosmotic flow of analytes and the potential at the bipolar electrode. Electrochemiluminescence was achieved in the reporter channel using luminol/H_2O_2 to detect a luminescence signal. Several parameters were investigated to determine the effect on the ECL detector response including electroosmotic flow, reactant/co-reactant concentrations, electrode alignment, separation buffer optimization, and applied voltage using model analytes.

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

#49 – Progress Toward a Multifunctional Tetrode Sensor Probe: Validation of Pinnacle Technology's Glucose and Lactate Biosensors

Karissa Wilson[1,2], Jacob Theismann[1,3], Sam Kaplan[4], David Johnson[4], Daniel Allion[4], Michael Johnson[1,3]

[1]Department of Chemistry, University of Kansas, Lawrence, KS, USA; [2]Bethel College, North Newton, KS, USA; [3]Ralph N. Adams Institute for Bioanalytical Chemistry, University of Kansas, Lawrence, KS, USA; [4]Pinnacle Technology Inc., Lawrence, KS, USA

Lactate and glucose are molecules that play fundamental roles in cell metabolism. However, their relation to cognitive function is still not fully known. Biosensors used in vivo represent an important analytical tool for sorting out the roles these molecules play in cognitive performance. In collaboration with Pinnacle Technology, we are evaluating multifunctional sensors that will enable researchers to investigate these roles. We have conducted a series of experiments aimed at validating lactate- and glucose-selective biosensors produced by Pinnacle Technology. Our studies show that these sensors respond selectively to their respective analytes during calibration. In brain tissue, lactate sensors were able to detect changes in lactate concentrations; however, glucose measurements in brain tissue were complicated by cellular uptake mechanisms. We will describe these results in more detail and address alternative approaches to validate these sensors further.

#50 – Developing Tools to Study Cholesterol Metabolism in Microglia During Demyelination

Matthew C. Zupan and Meredith Hartley

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

Microglia are innate immune system macrophages that play a crucial role in central nervous system remyelination, in part by clearing myelin debris following demyelination. Myelin debris clearance is an essential process that allows for the recruitment of oligodendrocyte progenitor cells to the lesion site to initiate myelin repair. Thyroid hormone is known to induce phagocytosis in microglia. Thyroid hormone

agonists can promote remyelination in mouse models of myelin damage by promoting differentiation of oligodendrocyte precursor cells, but these drugs may also be acting on microglia to help promote remyelination. Our lab is interested in further exploring the role of thyroid hormone action in microglia and elucidating a novel mechanism for how thyroid hormone agonists promote remyelination. We have preliminary data showing that Sob-AM2, a CNS-penetrating thyroid hormone agonist, increases the phagocytic activity of primary microglia. In addition, we have data from animal models showing that Sob-AM2 treatment can reduce the accumulation of cholesterol esters in microglia during demyelination. To follow up on these results, we are currently developing a mass spectrometry assay that will allow us to study cholesterol metabolism in microglia. We will introduce isotopically labeled cholesterol and cholesterol ester to primary microglia and use mass spectrometry to analyze the resulting metabolites produced by the cells under different experimental conditions. We expect that microglia treated with thyroid hormone agonists in the presence of myelin should activate phagocytic pathways in microglia, therefore stimulating cholesterol metabolism and decreasing the accumulation of cholesterol esters. We are also studying Sob-AM2 action in microglia in a mouse model of demyelination in which the oligodendrocyte progenitor cells are not able to remyelinate. This will allow us to isolate the effect of Sob-AM2 on microglia from the effects of Sob-AM2 on oligodendrocyte progenitor cell differentiation. We expect that Sob-AM2 treatment will trigger microglia towards an anti-inflammatory, phagocytic, restorative phenotype and improve the neurological clinical signs of these mice, even in the absence of remyelination. Overall, we hope to better understand the role of thyroid hormone action on microglia to enable the development of better treatments for demyelinating diseases.