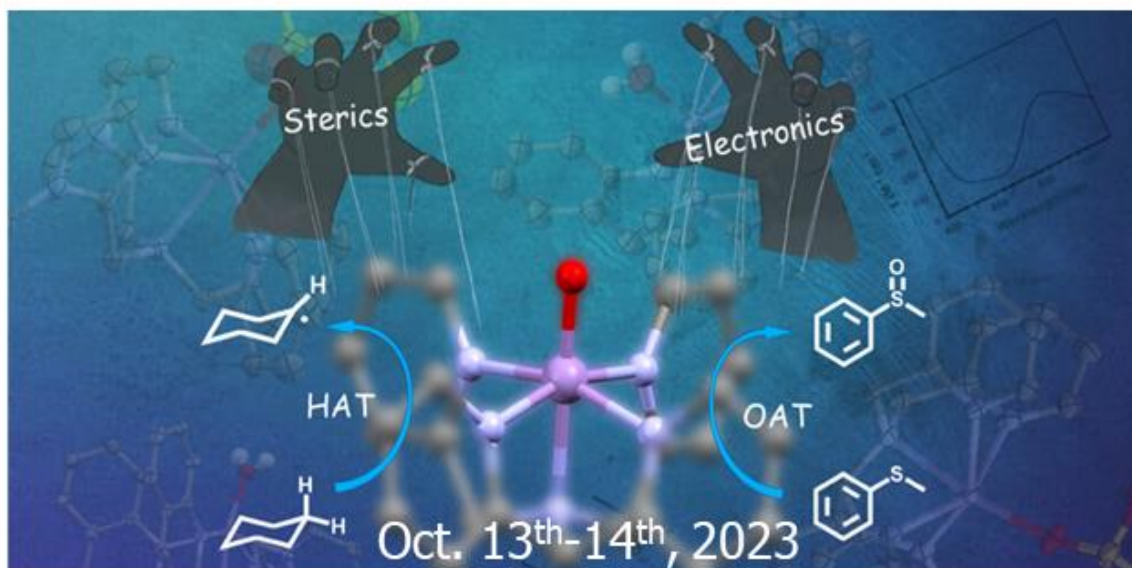
2nd Annual Chemical Biology Regional Symposium



Scientific Program and Abstracts

KU | NIH Center of Biomedical Research Excellence (COBRE)
Chemical Biology of Infectious Disease

KU | College of Liberal Arts & Sciences and School of Pharmacy
Graduate Training in Chemical Biology

KU | Office of Research

Scientific Program

Friday, October 13th – 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
James Martinez, Session Chair

Dr. Robert Cichewicz – Department of Biochemistry and Chemistry; University of Oklahoma

"*Assessing continental-scale fungal natural product diversity to enhance bioactive lead discovery*"

6:00 – 7:00 Dinner (Buffet)

7:00 Lightning Talks! – Graduate Trainees
Lexie Cutter, Session Chair

Alec Bevis, Department of Molecular Biosciences, Orozco Lab

"*Impact of PTPN22 and its autoimmunity-associated minor allele during coronavirus infection*"

Grahmm Funk, Department of Pharmaceutical Chemistry, Kim Lab

"Discovery of oral vaccine adjuvant via bioanalytical HTS assays"

Sayuri Niyangoda, Department of Chemistry, Johnson Lab

"Quantitation of zinc photo-release using fast scan cyclic voltammetry in zebrafish whole brain"

Matthew Russolillo, Department of Medicinal Chemistry, Farrell Lab

"*New chemical tools to study protein-carbohydrate interactions*"

Naviya Schuster-Little, Department of Chemistry, Whelan Lab

"Affinity-free enrichment and mass spectrometry characterization of the ovarian cancer biomarker CA125 (MUC16) from ascites and serum"

Saturday, October 14th – School of Pharmacy (SOP)

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

Check-in, Coffee, Juice, & Muffins

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

Welcome and brief opening comments – Symposium Organizers

L. Caetano M. Antunes – Assistant Professor, KU Department of Molecular Biosciences
"*Modulation of enteric pathogen virulence by microbiome derived-small molecules*"

Robin Orozco – Assistant Professor, KU Department of Molecular Biosciences
"*Autoimmunity and Infection: Understanding how the autoimmunity-associated allele of PTPN22 impacts anti-viral immune responses*"

Meredith Hartley – Assistant Professor, KU Department of Chemistry
"*Lipid Regulation during CNS Myelin Damage and Repair*"

Jingxin Wang – Assistant Professor, KU Department of Medicinal Chemistry
"*Development of RNA-degrading chimeras*"

10:45 – 11:00 AM Break

11:00 – 12:00 PM Special Speaker – 2020 SOP
Matthew Russolillo, Session Chair

Susan Lunte, KU Departments of Chemistry and Pharmaceutical Chemistry
"*Bioanalytical applications of microchip electrophoresis*"

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

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

Pankaj Baral – Assistant Professor, Kansas State University, Division of Biology
"*Neuroimmune regulation of carbapenem-resistant Klebsiella pneumoniae lung infection*"

Hyunjoon Kim – Assistant Professor, KU Department of Pharmaceutical Chemistry
"*TLR7/8 agonist-Nanomedicine for NK cell-mediated cancer immunotherapy*"

Prasad Dandawate – Assistant Professor, KU Medical Center
"*Acid ceramidase-1 is a potential drug target for inhibiting pancreatic cancer progression*"

2:00 – 2:15 Break

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

Scott Weir – Professor, KU Medical Center
"*The Institute for Advancing Medical Innovation – product development-focused translational research in an academic setting*"

Brian Sanderson – KU Data Science Core Lab
"Support for genomic data science at KU"

Jacob Kroh – Chemical Biology Trainee
"*Investigating a novel membrane interaction in the N-terminal domain of the Shigella major translocase IpaB*"

3:30 – 5:00 PM Poster Session (Snacks and Drinks), 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

Keynote Lecture – Friday, October 13th

Dr. Robert Cichewicz

Regents' Professor and INPART Director
Natural Products Discovery Group (NPDG)
Department of Chemistry and Biochemistry
University of Oklahoma
Norman OK 73019 USA

Assessing Continental-Scale Fungal Natural Product Diversity to Enhance Bioactive Lead Discovery

Fungi are the source of multitudes of chemically unique scaffolds. In many cases, these fungal derived compounds have been successfully translated into therapeutically useful molecules that have had tremendous positive impacts on human health and wellbeing. With only a small proportion of the Earth's fungi studied, it is reasoned that fungi will continue to yield new and useful compounds for many decades to come. This leads to a simple, yet challenging question concerning where and how researchers should approach the task of finding hidden molecular gems throughout the vast world of fungal natural products. To address this question, it is essential to first understand where and what natural products are available within the fungal Kingdom. Examining the distribution and diversity of fungal metabolites over continental-scale ranges can not only reveal new bioactive substances, but it can also help address gaps in scientific understanding of natural product evolution and diversity. Such knowledge is anticipated to illuminate paths to the discovery of chemical "dark matter" lurking in the distant corners of the natural products world, as well as provide practical tools for enhancing bioassay-guided fungal metabolite research processes. Examples of approaches to more efficiently capturing wider swaths of fungal natural product chemical diversity and efforts to apply those discoveries toward unmet human medical needs will be presented.

<u>Biographical Summary</u>

Robert joined the faculty at the University of Oklahoma in 2005 where he works as a Professor of Chemistry and Biochemistry and Director of the Institute for Natural Products Applications and Research Technologies (INPART). His lab, the Natural Products Discovery Group (NPDG), focuses on the identification of bioactive compounds with therapeutic applications from fungi, as well as developing new tools for discovering them. His group's work is supported by a highly successful citizen-science-based initiative that has enabled studies utilizing fungi from across much of North America.

Lightning Talks – Friday, October 13th

Impact of *PTPN22* and its Autoimmunity-Associated Minor Allele During Coronavirus Infection

Alec M. Bevis[1,2], Anam Shaikh[1,2], Catherine Kerr[1,2], Jenna Barnes[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*
[2]*The Office of Graduate Studies, University of Kansas, Lawrence, KS*

Allelic variation can impact the outcome of viral disease. Mice expressing the autoimmune-associated allelic variant of *Ptpn22* can clear Lymphocytic choriomeningitis virus clone 13 (LCMV-cl13) infection, but little is known regarding its impact during other virus infections. This research investigates how the loss of *Ptpn22* (PEP-null) and its minor allelic variant (PEP-619WW) impacts antiviral immunity during coronavirus infection**. We hypothesize that PEP-null and PEP-619WW mice will have enhanced antiviral immunity during coronavirus infection.** This hypothesis was tested using Mouse Hepatitis Virus (MHV) A59 in our PEP-WT, PEP-null, and PEP-619WW mouse models. Following infection, PEP-null and PEP-619WW mice have reduced weight loss, viral titer, and increased survival over PEP-WT mice. Next, we determined if lymphocytes were necessary for disease recovery using lymphocyte-deficient Rag1^{-}/- PEP-WT and Rag1^{-}/- PEP-619WW mice. We show that PEP-619WW innate cells mediate some protection against MHV A59, but T and B cells are necessary for survival. Additionally, we investigated MHV A59 viral tropism and IFN-I production using PEP-WT, PEP-null, and PEP-619WW bone marrow-derived macrophage (BMM) and dendritic cell (BMDC) cultures. These experiments show that *Ptpn22* does not mediate viral permissiveness or IFN-ß production. The results described above demonstrate that PEP-619WW is beneficial during coronavirus infection and sets the precedent to interrogate its role in other RNA virus infections.

Discovery of Oral Vaccine Adjuvant via Bioanalytical HTS Assays

Grahmm A. Funk[1], Anuradha Roy [2], Hyunjoon Kim[1]

[1]*Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS*
[2]*IDAD/HTS Laboratory, University of Kansas, Lawrence, KS*

Purpose: Immunotherapy serves to modulate the host immune system to fight foreign pathogens or suppress autoimmunity. Conventional immunotherapies utilize parenteral injections, with limited utilization of oral delivery (e.g., polio vaccine). This administration, while effective, requires healthcare personnel for intradermal, intramuscular, or subcutaneous injection, which can present unique logistic challenges.[1] Additionally, traditional therapies induce systemic humoral immune response (IgG), but are less effective at inducing mucosal immune responses (IgA), which are critical to clear pathogens and reduce autoimmune activation in the lung and GI tract.[2] This study aims to discover and validate new compounds capable of selectively activating immune cells without triggering severe inflammation in the GI tract. Furthermore, we want to show these compounds are effective in modulating mucosal immunity and to create an efficient oral vaccine delivery platform.
Methods: High-throughput screening (HTS) of three drug libraries including FDA-approved compounds (FDA), soluble diversity (SDIV), and bioactives (BA) was employed for initial screening. Testing was done with RAW 264.7 Blue cells, utilizing a RAW-NF-kB promotor-SEAP assay. Quantification of SEAP in each well at 620nm was measured and compared to DMSO negative controls to identify initial "hits". Subsequent dose- and time-dependent testing of "hit" compounds was done to measure potency

and kinetics. Further validation of "hit" compounds was done by exposing mouse bone marrow-derived dendritic cells (BMDCs) to various concentrations of "hit" compounds. Both IL-10 and TNF-α cytokine production was quantified via ELISA, while immunophenotyping of the BMDCs was performed by flow cytometry analysis. Finally, cytotoxicity of "hit" compounds was assessed via an LDH assay against Caco-2 cells.

Results: Initial screening yielded a total of 117 "hit" compounds across all three libraries screened. In addition, several other compounds showed significant reductions in SEAP absorbance opposed to increases. Average minimum dose required for SEAP activation for "hits" was 5 μM. Time-dependent SEAP activation was greatest for "hits" and LPS controls at 0.5h, decreasing with longer exposure time. Several compounds demonstrated the ability to stimulate either pro- or anti-inflammatory cytokines and/or increase the expression of CD40 or CD80 above non-treated controls. Caco-2 cell viability of "hit" compounds was minimal at 1 μM (< 88%), but two compounds tested showed reduced cell viability at concentrations of 10 μM (<75%).

Conclusions: Initial discovery of potential new immunomodulatory drugs is crucial to develop increasingly potent and targeted immunotherapies. Expansion of traditional HTS screening with the inclusion of dose- and time-dependent activation helped to narrow "hit" compound selection criteria. In the larger picture, these compounds could serve as an oral vaccine adjuvant to overcome challenges associated with developing prophylactic and therapeutic regimes for GI tract infectious disease and autoimmune issues. Future work of this study will aim to formulate these compounds into poly(lactic-co-glycolic) acid (PLGA) nanoparticles for use as an oral vaccine designed for GI delivery.

References: 1. J. N. Zuckerman, et al. *Bmj*. 321 (2000), pp. 1237–1238. 2. A. T. Jones, et al. *Nat. Commun*. 10, 1–14 (2019).

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

Quantitation of Zinc Photo-release using Fast Scan Cyclic Voltammetry in Zebrafish Whole Brain

Sayuri S. Niyangoda[1], Jodie Kearney[1,2], Jacob Theismann[1], Michael A. Johnson[1]

[1]Department of Chemistry and R.N Adams Institute for Bioanalytical Chemistry, Lawrence, KS; [2]School of Biotechnology, Dublin City University, Dublin, Ireland

Being the second most abundant transition metal ion in the human brain, free ionic zinc is known to be involved in numerous physiological functions including the modulation of neurotransmission in the brain. The use of caged zinc compounds photoactivated to allow application of zinc ions into the brain with small spatial and temporal resolutions, has revealed that photo-released zinc ions resulted in decreased dopamine reuptake. However, a limitation in this approach is the difficulty in quantifying the amount of zinc that induced this effect. Here, we have addressed this issue by developing an indirect approach to determine the amount of caged zinc ions released, by using fast scan cyclic voltammetry at carbon fiber microelectrodes and flow-injection analysis to optimize a waveform that is sensitive to 4-methoxy-3-nitrobenzaldehyde, which is the electroactive byproduct of the photoreaction. This optimized sawhorse waveform employed a switching potential of 1.4 V, accumulation potential of -0.6 V, a scan rate of 600 V/s and a 3 ms holding potential. This approach yielded a linear relationship between the byproduct concentration and oxidation current, and the electrodes did not foul upon multiple injections. Furthermore, the biological application of the method was demonstrated by measuring the released zinc ions that was evoked by light exposure in whole brains of adult zebrafish. Photocaged zinc exposed to 200 milliseconds of light exposure, released zinc in the mid-nanomolar range during experimentation. This method can

provide a foundation to explore the role of zinc in different pathway specific modulations during neurotransmission. The ability to quantify the spatial and temporal availability of zinc ions could be particularly beneficial in understanding the role of zinc in disease conditions such as Parkinson's disease and Alzheimer's disease.

New Chemical Tools to Study Protein-Carbohydrate Interactions

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

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

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

Affinity-free Enrichment and Mass Spectrometry Characterization of the Ovarian Cancer Biomarker CA125 (MUC16) from Ascites and Serum

Naviya Schuster-Little[1], Roberta Fritz-Klaus[2], Andrew Sokolovsky[1], Mark Etzel[3], Niharika Patankar[2], Saahil Javeri[2], Manish S. Patankar[2] and Rebecca J. Whelan[1]

[1]Department of Chemistry, University of Kansas, Lawrence, KS
[2]Department of Obstetrics and Gynecology, University of Wisconsin-Madison, Madison, WI, USA
[3]Department of Food Science, University of Wisconsin-Madison, Madison, WI, USA

CA125, an FDA-approved biomarker for ovarian cancer, is a peptide epitope on the glycoprotein MUC16 detected via immunoassay. The epitope constitutes a small percentage of MUC16 and may not be presented on every molecule, resulting in undercounted CA125 levels. We hypothesize that a proteomic assay using mass spectrometry will detect regions of MUC16 that are not detected in the immunoassay. We have developed a workflow to enrich MUC16 from biofluids using filtration, ion exchange, and size-exclusion chromatography. This process enriches MUC16 from large volumes of ascites (peritoneal fluid) without introducing bias possible with antibody-based methods. Following enrichment, we performed bottom-up proteomics and detected 2 – 53 MUC16 peptides in ascites samples from three ovarian cancer patients. Having achieved successful enrichment of MUC16 from ascites, we are miniaturizing the process to use volumes of serum compatible with standard clinical blood draws. This miniaturized workflow uses filtration, depletion of abundant serum proteins, and Q-Nano ion-exchange membrane chromatography. We have enriched MUC16 from serum that contain CA125 counts below the clinical cutoff. Future work is focused on bottom-up proteomic analysis of MUC16 enriched from serum. In addition to the bottom-up proteomics approach, we are developing a targeted mass spectrometry assay that should enable greater specificity for MUC16 and lower limits of detection compared to both the clinical immunoassay and our current bottom-up proteomics approach. An assay like this is important because serum CA125 levels are

routinely monitored in patients in remission after ovarian cancer treatment, and rising CA125 levels correlate with cancer recurrence. There is a need to develop a more sensitive assay to detect recurrence early, when treatment options are most effective. Our approach will meet this need in ovarian cancer clinical diagnostics.

Special Seminar – Saturday, October 14th

Bioanalytical Applications of Microchip Electrophoresis

Dr. Susan M. Lunte

Departments of Chemistry and Pharmaceutical Chemistry, University of Kansas, Lawrence, KS

Oxidative stress is involved in many neurological diseases including Alzheimer's Disease and traumatic brain injury. New tools capable of monitoring biomarkers of oxidative stress in vivo and in vitro can be used to investigate the cause and progression of these disease states. Microchip electrophoresis is a powerful tool for the analysis of biological samples. In particular, its ability to perform fast, efficient separations of multiple analytes in a single run makes it possible to monitor several biomarkers in a single sample with high temporal resolution. In addition, the small dimensions of the channels in the chip are compatible with the analysis of microdialysis samples and single cells. In this presentation, two applications of microchip electrophoresis (ME) for biochemical investigations will be presented. The first involves the development of ME-based methods for the detection of reactive nitrogen and oxygen species (RNOS) in macrophages and immune cells. This includes direct amperometric detection of RNOS as well as the evaluation of fluorescent reagents used for specific species. The second application involves the combination of microdialysis with microchip electrophoresis for near real-time continuous in vivo monitoring of biogenic amines and biomarkers of inflammation. The ultimate goal is to use these tools to investigate the role of oxidative stress in neurodegenerative disease.

Invited Speakers, Saturday October 14th

Modulation of enteric pathogen virulence by microbiome derived-small molecules

Dr. L. Caetano M. Antunes

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

In the Antunes Laboratory, we are broadly interested in the roles played by small molecules during host-microbiota-pathogen interactions. To date, we have focused on the impact of microbiome-derived small molecules on enteric pathogen behavior. We have previously shown that the human gut harbors thousands of small molecules, most of which are yet to be identified. Organic extracts of human feces have been used to determine pathogen responses to the chemical milieu of the human gut. Our results show that multiple enteric pathogens, such as Salmonella enterica, Vibrio cholerae, and Clostridioides difficile display marked transcriptional responses to the human gut metabolome, and that genes required for host interactions are modulated. Bioactive microbiome members and compounds can be identified, and our ongoing work is focused on identifying novel compounds and revealing the molecular mechanisms behind bioactivity.

Autoimmunity and Infection: Understanding how the autoimmunity-associated allele of PTPN22 impacts anti-viral immune responses

Dr. Robin Orozco

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

The 1858C>T allele of PTPN22 is present in about 5-15% of the North American population and is one of the highest risk alleles in the development of numerous autoimmune disorders including Type I Diabetes, Rheumatoid Arthritis, and Lupus. PTPN22 is expressed exclusively in immune cells and regulates key immune response functions such as T cell receptor signaling, cytokine production, and macrophage polarization. Despite these PTPN22 regulated immune responses being critical for the anti-viral immune response, little is known about how this common, autoimmunity-associated allele impacts virus infection. Our lab addresses this research gap by using murine system where the Ptpn22 gene was mutated to express the equivalent autoimmunity-associated allele. Following infection with potentially persistent virus LCMV-cl13 Ptpn22-mutant mice can clear the infection, whereas wildtype mice cannot. This is paired with an enhanced T cell response and more pro-inflammatory dendritic cell phenotype in the Ptpn22-mutant mice. This talk aims to unravel the underlying immune based mechanisms of this phenotype, expands these studies to additional virus infections, and discusses the potential of therapeutically targeting Ptpn22 to overcome virus infection.

Lipid Regulation during CNS Myelin Damage and Repair

Dr. Meredith Hartley

Department of Chemistry, University of Kansas, Lawrence, KS

Myelin damage is a major feature of multiple sclerosis and is also observed in other neurological diseases including Alzheimer's, however, there are no FDA-approved therapies that directly target myelin repair. Formation of the lipid-rich myelin sheath is induced by hormones, and our goal is to define how CNS

lipids are regulated during neurological diseases with demyelination. We have used genetic mouse models of demyelination to profile lipid dynamics during disease. Brain, spinal cord, and plasma samples from distinct demyelinating and remyelinating phases were analyzed by mass spectrometry and the levels of over 300 distinct lipids were measured. Principal component analysis was used to demonstrate that the CNS lipidome has unique signatures during demyelination and remyelination. Volcano plot analysis was used to identify lipid classes and lipid species that differentially regulated during demyelination and remyelination. The analysis also revealed that remyelination in the spinal cord is greatly impaired relative to the brain. Our current research is focused on several areas. (1) Cholesterol esters were identified in the lipidomics panel as potential CNS biomarkers of myelin damage. We are currently performing experiments to identify which enzymes and proteins are involved in the regulation of cholesterol esters. Our goal is to better understand how cholesterol ester formation impacts the ability of the CNS to successfully remyelinate. (2) We are interested in exploring how different hormones (thyroid hormone and estrogen) impact lipid regulation during demyelination to better understand how hormones regulate CNS lipids during disease.

Development of RNA-degrading chimeras

Dr. Jingxin Wang

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

mRNA plays a pivotal role in genetic information transfer and has garnered attention as a potential therapeutic target for a wide range of diseases. However, the efficacy of RNA-binding small molecules or antisense oligonucleotides alone can be limited. To address this challenge, we have created RNA-degrading chimeras by covalently linking RNA ligands with ribonuclease-recruiting fragments. This innovative approach transforms inactive RNA binders into active RNA degraders with therapeutic potential. We have designed and optimized RNA-degrading chimeras that potently reduced RNA levels of the SARS-CoV-2 virus in cellular models. Additionally, we have developed an antisense-based RNA degrader targeting alpha-synuclein, a pathogenic gene in Parkinson's disease. Our research offers a fresh perspective on the design of gene expression inhibitors for future therapeutic applications.

Neuroimmune regulation of carbapenem-resistant Klebsiella pneumoniae lung infection

Dr. Pankaj Baral

Microbiology and Immunology Section, Division of Biology, Kansas State University

Carbapenem-resistant Klebsiella pneumoniae (CRKP) causes lung infections and fatal pneumonia-derived sepsis (or pneumonic sepsis) for which minimal treatment options are available. They are responsible for 20-40% mortality among the hospitalized patients. Host targeted alternative therapeutic approaches are thus necessary for pneumonia and sepsis. The respiratory tract is densely innervated by nociceptive sensory neurons that mediate pain production and release of neuropeptides in the lungs, including calcitonin gene-related peptide. CGRP acts on its cognate receptor complex (RAMP1/CALCRL) expressed in immune cells for immunomodulation. However, we do not yet understand the role of nociceptive neurons and CGRP in lung defenses to CRKP and pneumonic sepsis. Specifically, our research will address the following two key questions: 1) Do nociceptor neurons and their subsets play a role to alter host survival, bacterial clearance, and pathology in pneumonic sepsis 2) Do neuropeptide signaling involve in driving pneumonic sepsis? Using both 'loss and gain of function' neuronal manipulating strategies in mice and using the neuropeptide receptor deficient mice, we will study the neuroimmune

interactions in CRKP pneumonia to address these questions. Our preliminary in vivo and in vitro data demonstrate the host deleterious effects of nociceptive neurons and CGRP signaling pathway for the protection against lethal CRKP pneumonia. Further, the nociceptive-depleted mice showed higher recruitment of neutrophils (CD11b+Ly6G+) and inflammatory monocytes (CD11b+Ly6Chi) at 24h post-infection as compared to control littermates. Targeting the nervous system directly, or through downstream receptor signaling pathways in immune cells, will inform about the host-based strategy as a treatment modality for pneumonia and sepsis.

TLR7/8 agonist-Nanomedicine for NK cell-mediated Cancer Immunotherapy

Dr. Hyunjoon Kim

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

Introduction: Monoclonal antibodies targeting specific tumor cell surface receptors, EGFR and HER-2, are approved for the treatment of various solid tumors. In addition to their direct inhibitory effects on these receptors, the antibodies also elicit natural killer (NK) cell-mediated antibody-dependent cell cytotoxicity (ADCC). Activation of NK cells requires stimulation by dendritic cells (DCs). In this study, we formulated immunostimulatory nanoparticles (ISNP) by encapsulating novel imidazoquinoline-based, mixed TLR7/8 agonist (termed '522') in poly(lactide-co-glycolide) (PLGA) nanoparticles. Our hypothesis is that ISNPs can induce DC activation and expansion of NK cells, which will result in enhanced ADCC.

Methods: ISNPs were fabricated using double emulsion solvent evaporation technique. We measured the size and the zeta-potential of the ISNPs using dynamic light scattering. Morphology of ISNPs was imaged by cryo-transmission electron microscopy. Release of 522 from ISNPs was determined in acidic pH (5.5, 6.5) and neutral pH (7.4). Immunocompetent C57bl/6 mice received ISNPs via intravenous (IV) or subcutaneous (SC) injection and NK cell activation was determined by flow cytometry. Peripheral blood mononuclear cell (PBMCs) were collected from healthy donors and NK cell activation was determined by CD25 and IFN-ɣ expression by flow cytometry. Soluble form of 522 (Free522) and TLR3 agonist polyinosinic:polycytidylic acid (poly I:C) were used as controls.

Results: ISNPs were spherical in shape and had an average hydrodynamic diameter of 202 nm. ISNPs showed acid-responsive release kinetics. In vivo, ISNPs increased number of activated NK cells by 3- and 2.5-fold compared to untreated group and Free522 group, respectively, following IV and SC administration. In human PBMC assays, ISNP treatment dramatically increased expression of CD25 and IFN-ɣ on NK cells; CD25 expression increased from 1.5% for vehicle treatment to 20% for ISNP treatment, while Free522 and poly I:C treatment resulted in 12% and 7% expression, respectively. IFN-ɣ expression also increased from 13% for vehicle treatment to 41% for ISNP treatment and Free522 and poly I:C treatment resulted in 31% and 23%, respectively.

Conclusion: The new TLR7/8 agonist 522 was successfully encapsulated in acid-responsive PLGA nanoparticles. The free agonist induced stronger NK cell activation than poly I:C and encapsulation in nanoparticles further enhanced NK cell activation. These results suggest that ISNPs can be a potent adjuvant for NK cell-mediated cancer immunotherapy.

Acid ceramidase-1 is a potential drug target for inhibiting pancreatic cancer progression

Dr. Prasad Dandawate

Department of Cancer Biology University of Kansas Medical Center, Kansas City, KS

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer-related deaths in the United States. PDAC is an aggressive disease characterized by poor prognosis, rapid progression, drug resistance and recurrence after surgery. The current chemotherapy treatment is insufficient to improve survival to <10%. Altered sphingolipid metabolism (SM) was identified as a hallmark of cancer. The major sphingolipid metabolites are ceramide, sphingosine and sphingosine-1-phosphate (S1P). The sphingolipid metabolites ceramide (proapoptotic) gets converted to tumor-promoting sphingosine-1-phosphate (S1P) by acid ceramidase 1 (ASAH1) and sphingosine kinase (SPHK1/2). Ceramide is proapoptotic, while S1P induces proliferation and migration in cells. S1P levels are high in cancer cells, and it play a role in cancer progression. Hence, reducing the S1P production becomes a novel strategy for cancer treatment. We found that genes involved in SM are upregulated in PDAC. SPHK1 has been targeted in cancers, but kinase inhibitors are traditionally known for their non-selectivity and toxicity. Hence, there is a need to identify other targets to inhibit the production of S1P. Therefore, we decided to target ASAH1. Inhibiting ceramidase will inhibit sphingosine production and accumulation of ceramide in cells that can induce cancer cell apoptosis. Our analysis of the cancer genome atlas (TCGA) database showed that acid ceramidase 1 (ASAH1) is uniquely overexpressed in PDAC patients compared to normal individuals. Further, our western blot and IHC data showed that ASAH1 was overexpressed in PDAC tissue and cell lines. The knockdown of ASAH1 resulted in reduced proliferation, colony formation, migration and spheroid formation in PDAC cells, suggesting its role in tumor progression. Hence, to target ASAH1, we synthesized a series of analogs based on the ceritinib-2 motif (an established ASAH1 inhibitor). Of these inhibitors, two analogs, Cer1 and Cer3, effectively inhibited the proliferation of PDAC cells at nanomolar concentration. Further, Cer1 and 3 inhibited colony formation and induced cell cycle arrest and apoptosis in PDAC cell lines. Further, our molecular docking and cellular thermal shift assay data confirmed the specific binding of Cer1 and Cer3 to ASAH1 protein. Moreover, both analogs inhibited ASAH1 enzyme activity, production of S1P and increased ceramide levels in PDAC cells. We are currently evaluating the anticancer activities of these compounds in an orthotopic model of PDAC cells in mice. Overall, ASAH1 is overexpressed in PDAC and plays a role in tumor progression. Our novel ceritinib-2 analogs block ASAH1 in PDAC cells and inhibit their proliferation, which induces apoptosis. Hence, targeting ASAH1 represents a novel strategy to inhibit PDAC progression by inhibiting sphingolipid metabolism and S1P production.

The Institute for Advancing Medical Innovation – Product Development-Focused Translational Research in an Academic Setting

Dr. Scott Weir

Director, Institute for Advancing Medical Innovation, University of Kansas Medical Center

Associate Director – Translational Research, University of Kansas Medical Center

The traditional role of academic investigators in the discovery and development of new therapies has been to generate fundamental biology discoveries to better understand how diseases emerge and progress rather than to conduct applied or translational research focused on how to treat, prevent, or control diseases. The role of academia, however, has dramatically changed over the past 25 years. The majority of new therapies approved by the US FDA between 1985 and 2009 arose from NIH-funded academic research. Each of the 210 new molecular entities approved by the FDA from 2010-2016 were associated with NIH-funded research. With the increasing contribution of academic research to the successful development of new therapeutics, academia-industry collaborations are becoming even more important as we continue to progress through the 21st Century. Further, industry is increasingly requiring universities and academic investigators to advance medical innovations further along the translational research path prior to

partnering. In 2006, the University of Kansas, partnering with the Ewing Marion Kauffman Foundation, established the Institute for Advancing Medical Innovation (IAMI). IAMI serves as the product development-focused translational research enterprise for the University and its regional partners. IAMI partners with researchers to translate laboratory and bedside discoveries into therapeutic, diagnostic, and medical device technologies, and using an industry approach, executes product development-focused translational research designed to de-risk the technologies with the intent of partnering those with promise. Empowered multidisciplinary, multi-organizational project teams develop milestone-based product development-focused translational research project plans and are mentored through project execution. IAMI has evaluated over 200 KU investment opportunities, investing over $10M in 63 projects. To date, twelve investments have been partnered with the private sector, with one drug product on the market. Over the past six years, IAMI has supported advancement of five KU-invented anticancer agents to early phase clinical trials and currently supports a cancer drug discovery portfolio of 30 projects. This presentation describes how IAMI partners with investigators to establish new projects, and through an innovative public-private partnership, CureBridgeCollaborative®, how novel medical innovation technologies advanced from the bench to the patient's bedside.

Support for Genomic Data Science at KU

Dr. Brian Sanderson

Data Science Core Lab, University of Kansas, Lawrence, KS

Recent and continuing advances in biotechnology have facilitated tremendous progress in the acquisition of biological information in the form of sequence and image data. Effective use of these data requires integration of data engineering, software design, and statistical analysis, and each of these domains depends on specialized training. The Genomic Data Science Core is a new analysis and training service at KU, funded by the Kansas INBRE Program and the KU Center for Genomics Research Rising. We will provide an overview of our mission, highlight some recent analysis projects to give examples of our workflows and the services and products we can provide, and describe the process of initiating collaborations with our team.

Investigating a Novel Membrane Interaction in the N-Terminal Domain of the Shigella Major Translocase IpaB

Jacob Kroh

Chemical Biology Trainee, Department of Molecular Biosciences, University of Kansas, Lawrence, KS

The Type III Secretion System (T3SS) is a needle-like protein macromolecular machine used by many Gram-negative bacterial pathogens such as members of the *Shigella, Salmonella, Burkholderia, Yersinia*, and *Pseudomonas* families that allows them to infect eukaryotic cells. The T3SS is composed of numerous proteins that form a macrostructure that has three components: the needle-like injector apparatus composed of the inner and outer membrane rings, needle, tip, and translocon. Effector proteins of diverse function, and chaperone proteins that bind the effectors and guide them to the needle complex. The T3SS translocon is a complex formed by two membrane proteins -- the major translocon protein and the minor translocon protein. In *Shigella*, the major translocon protein is IpaB, and the minor translocon protein is IpaC. IpaB is predicted to have two transmembrane domains. Additionally, regions of this protein are expected to be associated with the membrane. Exactly where these regions are, and how they will interact with the membrane is poorly understood.

At the moment, there are predictions that IpaB will have transmembrane regions somewhere between residues 313-333 and 399-419 . However, there is currently no experimental data in the literature that supports these predictions, or that shows a direct interaction between any IpaB residue with the membrane. We here report a pair of membrane interactions in the N-terminal domain of IpaB. We used site directed mutagenesis to engineer cysteine point mutations in IpaB, expressed and purified the cysteine mutants, and labeled the proteins with a fluorine tag BTFA (3-bromo-1,1,1,-trifluoroacetone). We acquired 1D 19F NMR of the BTFA-labeled proteins in the presence and absence of micelle, and determined which residues showed changes in the 19F NMR peaks in a membrane mimic. Our results suggest a possible use of 19F NMR to map the membrane interactions of the different domains of IpaB and opens the door to future work on characterizing the T3SS translocon proteins.

Posters

#1 – Development of an Improved Transposon Mutagenesis System for *Chlamydia*

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Chlamydia is an obligate intracellular pathogen the most frequent bacterial sexually transmitted infection and the leading cause of infectious blindness worldwide. *Chlamydia* has a highly reduced and conserved genome with limited metabolic and biosynthetic capabilities. A saturated mutant library would be a valuable tool to probe which metabolic processes are critical versus dispensable for *Chlamydia* growth and pathogenesis. Transposon based genetic disruption systems using repeated transformations with suicide vectors have been developed for *Chlamydia*. However, transformations are inefficient and time-consuming in *Chlamydia,* limiting the number of mutants that can be feasibly generated. This study aims to develop a replication-competent, inducible transposon mutagenesis system, eliminating the need for multiple transformations. Our data show that the transposon and the transposase under the *Tet* inducible system cannot be stably maintained on the same plasmid, likely due to leaky expression of the transposase prematurely mobilizing the transposon. To control when the transposon can be mobilized, we have developed and transformed into *Chlamydia* separate plasmids carrying either the transposase or the transposon. We are attempting lateral gene transfer experiments via co-infection of two *Chlamydia* strains with (1) the plasmid encoding a transposon and (2) the plasmid encoding a transposase. Preliminary data show that transposase induction during the coinfection does not result in a significant reduction in progeny, indicating that the transposon has not been mobilized into the genome. Further work will use unique fluorescent markers to monitor the sharing of these plasmids between strains and a FLAG tag to monitor expression of the transposase.

#2 – Measurement of Total Reactive Oxygen Species Production in Human Microglia Using Microchip Electrophoresis with Laser-Induced Fluorescence Detection

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Oxidative stress resulting from excessive production of reactive oxygen species (ROS) by overactive pro-inflammatory microglia is thought to contribute significantly to the pathogenesis and progression of Alzheimer's disease (AD). Reduction of inflammation and oxidative stress induced by microglia is therefore a potential therapeutic strategy for AD treatment. Evaluating the efficacy of potential therapeutics requires analytical methods capable of reliably measuring levels of ROS within microglia, which remains challenging due to their instability and short half-life. One approach to overcome this issue is by using the fluorogenic probe, 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA), which forms a highly fluorescent species, DCF, upon reaction with several ROS. The work presented here focuses on the development of a method utilizing H2DCFDA to measure changes ROS production in a human microglial cell line, HMC3, in response to stimulation with amyloid beta. HMC3 cells cultured in the presence and absence of amyloid beta were harvested and loaded with H2DCFDA. Cell samples were then lysed, diluted in electrophoresis buffer containing a fluorescent internal standard (6-carboxyfluorescein) and analyzed

using microchip electrophoresis with LIF detection (ME-LIF). Optimization of separation conditions, such as buffer composition and separation distance will be discussed, as well as preliminary results.

#3 – Subcloning of various constructs of the Shigella virulence protein IpaC for protein expression

Thomas Bear and Roberto N. De Guzman

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Type III Secretion System (T3SSs) is a complex system utilized by many Gram-negative bacteria as a mechanism for pathogenesis. For the T3SSs the translocon is a portion of the complex that spans through the hosts cell membrane to create a pore for the insertion of virulence factors into the host cell. The structure of the translocon for both major and minor components has not been determined due to lack of imaging for its structure. Isolation of different portions of the transmembrane domain of the translocon is needed to identify which residues are critical for the formation of the trans-membrane domain of the T3SS. I aimed to create expression vectors for the IpaC N and C terminus and express them in pET-22b. The plasmids were successfully inserted into pET-22b and confirmed via DNA sequencing.

#4 - Developing Novel Antimicrobials Against Multidrug Resistant *Acinetobacter baumannii*

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Acinetobacter baumannii is an emerging nosocomial pathogen that causes a variety of infections, ranging from minor soft tissue to life-threatening infections. The severity and types of infections depend on multiple factors, including the genetic and phenotypic variations of the strains. The pathogen has the ability to acquire antibiotic resistance genes from the environment. This trait has allowed the organism to persist in healthcare settings and has also facilitated the global emergence of multidrug resistance (MDR). *A. baumannii* is becoming resistant to most of the commonly used antibiotics. MDR strains pose challenges for clinicians treating infections caused by these strains. The development of drug resistance also imposes additional economic burdens on the healthcare system. Thus, there is an urgent need for the development of novel strategies to control infections caused by *A. baumannii.* Using a high-throughput screening assay, we recently examined a small-molecule library called CMLD at the University of Kansas. During this study, we identified several potential candidates that either specifically inhibited *A. baumannii* strains or displayed broad-spectrum inhibitory activity against *A. buamannii.* In this presentation, we will be showcasing our results on two anti-infective molecules that specifically inhibit *A. baumannii.* We present our findings regarding inhibitory spectra, mode of action, and synergistic activities with other antibiotics.

#5 - Development and Characterization of a New Mn^{III}-alkylperoxo Complex

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Inspired by various manganese dependent enzymes, a range of synthetic manganese catalysts have been developed to carry out a variety of highly stereo-selective oxidation reactions such as olefin epoxidations. In these synthetic catalysts, the proposed catalytic mechanism invokes the heterolysis of the oxygen-oxygen bond of a Mn^{III}-hydroperoxo intermediate to generate the active oxidant - a manganese(V)-oxo species. However, the factors controlling this critical O-O activation step remain poorly understood. Work by our group and others has utilized Mn^{III}-alkylperoxo complexes to mimic the important Mn^{III}-hydroperoxo intermediates. From these investigations, there has been evidence that Mn^{III}-alkylperoxo complexes can decay by O-O homolysis and heterolysis pathways. Presumably, perturbations in the ligand-sphere of these Mn^{III}-alkylperoxo complexes could be employed to modulate the decay process. To that end, a new Mn^{III}-alkylperoxo complex has been developed to investigate the effect of perturbations on the mechanism of cleavage of the oxygen-oxygen bond. A series of combined computational and kinetic investigations was completed with the goal of probing the effects of such a change to the ligand-sphere on the decay mechanism. These studies have allowed us to gain a further understanding of the control of the ligand sphere over the reactivity of Mn^{III}-alkylperoxo adducts.

#6 - A Series of SARS-CoV-2 Mac1 Pyrrolo-Pyrimidine-Based Inhibitors Repress Coronavirus Replication in Cell Culture

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Coronaviruses (CoVs) are well-known to emerge from zoonotic sources and cause severe human and veterinary diseases, including recent outbreaks of MERS-CoV and SARS-CoV-2. All Coronaviruses (CoVs) encode for macrodomain protein (termed Mac1) that binds to and removes ADP-ribose from protein. Mac1 is essential for CoV pathogenesis, though how it promotes virus replication is still widely unknown. Developing Mac1 inhibitors would be beneficial for identifying the functions of Mac1 and could be used to treat disease caused by CoVs. MHV-JHM is an ideal model for testing Mac1 inhibitors because Mac1 is essential for the replication of this virus. Previous screens identified a compound, MCD-628, that inhibited Mac1 activity *in vitro* at low micromolar levels. However, MCD-628 had no impact on virus replication, which we hypothesized was due to an acidic side chain that reduces its permeability. To explore this hypothesis further, we tested compounds with methyl and ethyl esters modifications at the same site, which could be converted back into MCD-628 by cellular esterases. We demonstrated through eADME that the methyl-ester version of MCD-628 had a greater Log_D value than MCD-628, indicating that it has higher lipophilicity and likely increased membrane permeability. Importantly, we found that the methyl- and ethyl-esters of MCD-628, and two additional derivates of MCD-628, inhibited MHV replication in cell culture without notable cytotoxicity. We will use additional biological assays to confirm these results and identify drug-resistant mutants, which could indicate potential mechanisms used by Mac1 to inhibit virus replication.

#7 - Summer Internship with Design-Zyme LLC: Production of a Self-Assembling OspC-Based Lyme Disease Vaccine

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Lyme Disease is the most common vector-borne disease in the United States and Europe. This infection is caused by *Borrelia* bacterium and transmitted to humans when bitten by a *Ixodes* tick harboring the pathogen. There is no Lyme Disease vaccine commercially available for use in humans despite the staggering number of reported cases yearly. Additionally, climate change is expanding the range of tick populations geographically and extending the seasonal exposure period. Thus, increasing the potential risk of incidence and broadening antigenic variation of Lyme Disease. There is a dire need to develop an effective, multivalent, and broadly protective vaccine. *Borrelia* outer membrane protein C (OspC) has been identified as an attractive vaccinogen due its critical role in transmission, immunodominance, and universal presence among Lyme disease isolates. However, a challenge with OspC is sequence variability between *Borrelia* strains which limits broad protection from a single OspC variant.

Design-Zyme has developed a universal approach to rapidly create effective multi-valent vaccines against a broad range of species and variants. The approach allows a SpyCatcher003-Mi3 nanoparticle scaffold to spontaneous self-assemble with a SpyTag003 tagged single variant or multiple variants of interest.

During the internship, our goal was to couple Design-Zyme's Spycatcher003/SpyTag003-Mi3 Nanoparticle technology with OspC proteins to formulate two types of vaccines (1) A homotypic vaccine composed with the most common OspC variant to elicit an strong humoral immune response, and (2) a mosaic-8 multivalent heterotypic vaccine to elicit a cross-reactive antibody response against a greater range of strains of Lyme Disease.

I cloned, verified, and expressed all OspC variants needed to formulate the vaccines. Following expression, I efficiently purified each OspC variant via IMAC and SEC. The purified OspC proteins were incorporated with the Mi3-Nanoparticle for assembly. OspC protein production and purification played a critical role in creating the homotypic and heterotypic vaccines used to immunize *Borrelia* infected mice.

In a collaboration between the Hefty Lab and Design-Zyme, animal studies using the two vaccines demonstrated a strong humoral immune response against OspC. Challenge studies in mice demonstrated that the homotypic vaccine formulation (e.g., OspC sequence matches the strain used in challenge) was effective in providing protection, but that antibody titers were low against a range of OspC serotypes. The multi-valent vaccine also elicited a robust humoral immune response against all 8 OspC protein variants in mice and showed generation of antibodies against a broad range of serotypes, including those not present in the vaccine formulation. Challenge studies revealed protection with a single strain of *Borrelia*, but also suggested that vaccine dose and other immune responses need to be optimized to attain a broadly protective Lyme disease vaccine.

#8 - Binding of Group A Colicin T Domains to TolC

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The problem of antibiotic resistance poses a growing threat to human health, and as such necessitates the development of novel strategies to disrupt its various underlying mechanisms. One such mechanism in Escherichia coli involves the trimeric outer membrane porin TolC. TolC plays a central role for multiple bacterial efflux systems (i.e. AcrAB and MacAB), acting as the terminal pore through which antibiotics and other small molecules are pumped out of the cell. This modularity makes it an attractive target for the design of small molecule/peptide efflux inhibitors. Luckily, nature has provided a framework to probe the binding interactions of TolC via the bacteriocins – small, secreted proteins used to conduct both intra- and inter-species warfare. These proteins, known as colicins in E. coli, consist of an N-terminal translocation domain (T), a receptor binding domain (R), and a C-terminal cytotoxic domain (C). Several of these colicins bind TolC via their T-domains to facilitate the translocation of the toxic C-domain into the periplasmic space. We believe that structural characterization of the binding interactions between TolC and various colicin T-domains will help inform the design of small peptide efflux inhibitors to increase the efficacy of antibiotic treatment. In pursuit of this goal, we partnered with Dr. Emre Firlar and Dr. Jason T. Kaelber at Rutgers University to solve the structures of TolC in complex with the T-domains of Colicin E1 (ColE1), Colicin 5 (Col5), and Colicin E1* (ColE1*) using single-molecule cryo-electron microscopy.

#9 - Optimizing the Delivery of Antibody Therapeutics using Blood-Brain Barrier Modulators

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Monoclonal antibody (mAb) therapeutics are an important class of drugs used to treat a wide variety of brain diseases such as Alzheimer's disease (AD), multiple sclerosis, glioblastoma, and other brain cancers. However, the brain delivery of mAb therapeutics is challenging due to the presence of the blood-brain barrier (BBB). The tight junctions of the BBB are formed from protein-protein interactions, including VE-cadherin interactions, that seal the paracellular space. Thus, mAbs cannot cross the BBB into the brain through the paracellular route. The Siahaan laboratory has developed a novel method of modulating the BBB through the use of cadherin peptides that interfere with the cadherin-cadherin interactions to increase the porosity of the BBB paracellular pathway. Cadherin peptides (i.e., ADTC5, HAVN1) have been used to deliver a wide variety of molecules into the brain including ^{14}C-mannitol, gadopentetic acid (a magnetic resonance imaging (MRI) contrast reagent), 13.5 kDa 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 with BBB modulating peptides based on VE-cadherin (2) evaluate the safety of repeated BBB opening; and (3) evaluate the therapeutic efficacy of BDNF peptides co-delivered with E- and VE-cadherin peptides in an AD mouse model. Recently, two peptides based on VE-cadherin have been shown to increase the brain deposition of an anti-amyloid β mAb. In the future, the brain deposition of mAbs will be evaluated using a mAb tagged with a MRI contrast agent in a mouse model. Next, weekly administrations of HAVN1 and VE-cadherin peptides have been tested for their safety. In the future, giving multiple injections of the HAVN1 and VE-cadherin peptides at shorter intervals will be investigated. Finally, the efficacy of BDNF peptides will be evaluated when co-delivered with VE-cadherin peptides in an AD mouse model.

#10 - Role of PARP14 in HSV-1 Viral Replication

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Herpes simplex virus 1 (HSV-1) is a common linear double-stranded DNA virus that is estimated to infect 3.7 billion people worldwide. There is currently no vaccine or cure for HSV-1, and it can manifest itself as oral and facial sores in infected humans. The virus has two phases of infection: lytic or latent. Lytic infection occurs in epithelial cells and results in the expression of immediate early, early, and late proteins, ultimately leading to the production of infectious virus. Latent infection results in the viral genome being chromatinized and transcriptionally repressed for lytic viral genes in neurons of the sensory ganglia. Reactivation from latency occurs under conditions of stress, which initiates the lytic cycle. My research examines HSV-1-host interactions and focuses on poly-ADP ribose polymerases (PARPs) which are host proteins that have several functions, including the repair of damaged DNA, regulation of glycolysis, and restriction of viral replication. We specifically examined how HSV-1 replicates in the presence and absence of the PARP family member, PARP14, by monitoring intracellular and extracellular virus levels. Our preliminary data show that PARP14 knock-out cell lines have increased viral replication, in contrast to the control cell line that expresses PARP14. In the future, we will investigate whether PARP14 restricts viral gene expression, and the expression of type 1 interferons and interferon-stimulated genes. The rationale for studying HSV-1 and PARP14 is that our findings may be used one day to develop potential anti-HSV-1 therapeutics.

#11 - Development of RNA-Degrading Chimeras Targeting SARS-CoV-2 5' Untranslated Region

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RNA viruses such as SARS-CoV-2 feature highly structured untranslated regions (UTRs), which is vital for viral propagation. The indispensable roles of RNA structural elements in the UTRs of the viral genome make these RNA structures promising antiviral targets. In this work, we report a new class of coumarin derivatives that target the four-way junction RNA helix named SL5 in the 5' UTR of the SARS-CoV-2 genome. We optimized the coumarin derivative as a warhead and conjugated it to ribonuclease recruiting moieties to create RNA-degrading chimeras (RIBOTACs). We designed two different classes of RIBOTACs using an endogenous ribonuclease L recruiter 2',5'-linked oligoadenylate (2-5A) or its synthetic mimic D1. Although, the binding affinity between RNase L and D1 was demonstrated to be >10,000 times weaker than that of 2-5A, the RIBOTAC comprising D1 was found to be more active in degrading the SARS-CoV-2 RNA probably because D1 is electronically neutral under physiological conditions. The RIBOTACs robustly degraded SARS-CoV-2 RNA in cellular models at 1 μM and inhibited virus replication in lung epithelial cells at 20 μM. Our work provided important insight into designing RNA-degrading chimeras and their application in antiviral research. To discover the exact binding site of the coumarin derivatives, we developed a novel sequencing-based method called cgSHAPE-seq. First, we coupled a carboxylic acid imidazolide that can react with the hydroxyl group with the coumarin RNA ligand to direct the acylation on the 2'-OH group of the ribose at the ligand binding site. This covalent crosslinking then created a read-through mutation during reverse transcription at single-nucleotide resolution. After sequencing and mutational profiling, we unambiguously identified a bulged G as the only binding site of the coumarin-based ligand in the SARS-CoV-2 5' UTR, which was vigorously validated by mutagenesis and in vitro binding experiments.

#12 - Estrogen Depletion Effects on Lipid Homeostasis and Myelination

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Nearly 75% of multiple sclerosis patients are female and have symptoms that improve during pregnancy and worsen post-menopause, which coincides with changes in estrogen levels within the body.[1,2] Estrogen has been linked to neurological diseases, such as multiple sclerosis, which is a common neurodegenerative disease that involves the demyelination of axons within the central nervous system (CNS). Studies have shown that estrogens beneficial effects on myelination may be attributed to lipid changes in the CNS.[1,3] However, menopause causes a loss of estrogen, shows worsening symptoms in MS, and is associated with a decrease in glucose metabolism in the brain which leads to the breakdown of myelin lipids.[3] The regulation of lipids by estrogen signaling and menopause's effects on myelination and these lipid pathways have yet to be fully investigated. Therefore, an ovariectomized iCKO-*myrf* mouse model was used to mimic menopausal estrogen depletion. Ovariectomized and control mice underwent tamoxifen injections to induce demyelination before being tested for behavioral, histological, and lipidomic data. Rotarod and challenge ladder were used to monitor motor disability and recovery of mice. Brain and spinal cord tissue was collected from experimental mice at weeks 12 and 24 post tamoxifen injection, during peak demyelination and recovery, for BlackGold staining of myelin, electron microscopy imaging, and lipidomic analysis. These studies will serve to elucidate effects of menopause on myelination and lipids within the CNS and to characterize an iCKO-*myrf* mouse model of menopause.

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. Voskuhl, R.; Itoh, N.; Tassoni, A.; Matsukawa, M.; Ren, E.; Tse, V.; Jang, E.; Suen, T.; Itoh, Y., *PNAS*, **2019**, *116* (20), 10130.

#13 - Bringing Cryo-Electron Microscopy to Kansas and Beyond

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Over the past decade cryo-electron microscopy (Cryo-EM) has transformed the way we study the structure of macromolecules. Through improvements in microscope stability, development of direct electron detectors and new computational programs, researchers are now able to routinely solve high-resolution structures of previously understudied biomolecules. These recent advancements in structural biology have been collectively referred to as the "resolution revolution" and earned three pioneers within the field the 2017 Nobel prize in Chemistry. However, due the high price tag of the technology and extensive infrastructure required to house such instrumentation, regional access to cryo-EM facilities is severely limited. During the summer of 2023, KUMC acquired a new 200 kV Glacios cryo-EM equipped with a Selectris energy filter and Falcon 4i direct electron detector. The microscope is housed in the recently renovated Electron Microscopy Research Laboratory and is supported by two experienced full-time microscopists. In addition, the facility houses a sample preparation suite that is equipped with a TFS Vitrobot Mark IV as well as accessory equipment for freezing, clipping, and storing samples. The facility is further supported by the addition of a new top of line server-based analysis cluster that allows seamless

analysis, storage, and transfer of data collected on the Glacios microscope. The KUMC cryo-EM facility will allow investigators across the region to adopt cryo-EM into their research program by providing lab personnel training and access to a complete cryo-EM workflow.

#14 - *bar-1* and *egl-5* Independently Regulate Neuronal Specification of the DD6 and VD13 GABAergic Neurons

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Using fluorescent reporters of neuronal identity, genetic screens in *C. elegans* have elucidated genes that function in neuronal subtype specification. For example, in *C. elegans*, the 19 GABAergic motorneurons are formed in two waves, 6 DD-type form during embryogenesis, while 13 VD-type in larval stages. GABA neurons can be visualized using a differentiation marker, e.g., *Punc-25::gfp*. Transcription factors such as unc-30/PITX2 and cnd-1/NeuroD are required for DD and VD specification, in partially redundant fashion. Recently we found a fluorescent reporter, *Pplx-2::rfp* that is active in only VD13, the most posterior of the 19 GABAergic neurons. Expression of the reporter depends on canonical Wnt signaling, including *bar-1/b-catenin* and *egl- 5*/Hox-C. Loss of function in either gene results in reduced expression of *Pplx-2::gfp*, even though the cells are still GABAergic by *Punc-25::gfp*, while constitutive expression of *egl-5* in all GABA neurons induces expression of the RFP marker in 19 cells. Similarly, in DD neurons, a similar reporter, *Pflp-13::gfp* is expressed in DD1-5, but rarely in DD6. In *bar-1* and *egl-5* loss-of-function mutations, DD6 is GFP positive, and ectopic expression of *egl-5* silences *Pflp-13:gfp* in all DDs. In a recent genetic screen, we recovered a temperature-sensitive allele of *bar-1*, *lh41*. Using that we found that *bar-1* is required both for induction and maintenance of *Pplx-2* and *Pflp-13* expression. We will now mine available GABAergic neuron single cell transcriptomes to look for other cell-limited markers and determine if their expression also relies on *bar-1* and *egl-5*, or other Hox genes.

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

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

Recent highlights include the identification inhibitors of ACMS decarboxylase and DNAJA1 via virtual screening, using modeling to identify the functional activity of *Legionella pneumphila* effector protein SidI, using modelling to assess the structural impact of clinically relevant point mutations of TRIM32, modeling the interaction between the Type III secretion system basal body and sorting platform proteins SctK and SctD from *Pseudomonas aeruginosa*, and the optimization of an inhibitor of PTPRD.

With the software and expertise to perform virtual screening, protein-small molecule docking, protein/peptide modeling/docking, and cheminformatic analysis, the CCB is a valuable resource to enhance the productivity of researchers studying infectious diseases, in addition to other projects.

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#16 - Mutation of Highly Conserved Residues in Loop 2 of the Coronavirus Macrodomain Indicates that Enhanced ADP-Ribose Binding is Detrimental to Infection

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All coronaviruses (CoVs) encode for a conserved macrodomain (Mac1). Located in nonstructural protein 3 (nsp3), Mac1 is an ADP-ribosylhydrolase that binds and hydrolyzes mono-ADP-ribose from target proteins. Previous work has shown that Mac1 is important for virus replication and pathogenesis. Within Mac1, there are several regions that are highly conserved across CoVs, including the GIF motif. To determine how the biochemical activities of these residues impact CoV replication, the isoleucine and the phenylalanine were mutated to alanine in both recombinant Mac1 proteins and recombinant CoVs, including murine hepatitis virus (MHV), Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The F-A mutant proteins had ADP-ribose binding and/or hydrolysis defects that likely led to attenuated replication and pathogenesis in cell culture and mice. In contrast, the I-A mutations had normal enzyme activity and enhanced ADP-ribose binding. Despite increased ADP-ribose binding, most I-A mutant viruses were highly attenuated in both cell culture and mice, indicating this isoleucine residue controls ADP-ribose binding for efficient virus replication.

#17 – Using 19F NMR to Study Membrane Interactions of Shigella Type III Secretion System Translocon

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The Type III Secretion System (T3SS) is a needle-like protein macromolecular machine used by many Gram-negative bacterial pathogens such as members of the *Shigella*, *Salmonella*, *Burkholderia*, *Yersinia*, and *Pseudomonas* families that allows them to infect eukaryotic cells. The T3SS is composed of numerous proteins that form a macrostructure that has three components: the needle-like injector apparatus composed of the inner and outer membrane rings, needle, tip, and translocon. Effector proteins of diverse function, and chaperone proteins that bind the effectors and guide them to the needle complex. The T3SS translocon is a complex formed by two membrane proteins -- the major translocon protein and the minor translocon protein. In *Shigella*, the major translocon protein is IpaB, and the minor translocon protein is IpaC. IpaB is predicted to have two transmembrane domains while IpaC is predicted to contain one transmembrane region. Additionally, regions of these proteins are expected to be associated with the membrane. Exactly where these regions are, and how they will interact with the membrane is poorly understood. At the moment, there are predictions that IpaC will have a transmembrane region somewhere between residues 100-170. However, there is currently no experimental data in the literature that shows a

direct interaction between IpaC residues 100-170 with the membrane. Our goal is to characterize the membrane association of IpaC residues 100-170 using ^{19}F NMR methods. We additionally report an unexpected membrane interaction in the N-terminal domain of IpaB. We used site directed mutagenesis to engineer cysteine point mutations in both IpaB and IpaC, expressed and purified the cysteine mutants, and labeled the proteins with a fluorine tag BTFA (3-bromo-1,1,1,-trifluoroacetone). We acquired 1D ^{19}F NMR of the BTFA-labeled proteins in the presence and absence of micelle, and determined which residues showed changes in the ^{19}F NMR peaks in a membrane mimic. Our results suggest a possible use of ^{19}F NMR to map the membrane association of the different residues of IpaC and opens the door to future work on characterizing the T3SS translocon proteins.

#18 - Developing Sample Preparation and Capillary Electrophoresis Methods for the Analysis of Metabolites in Bacteria for Life Detection Applications

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Ocean worlds, such as Europa and Enceladus, have generated much attention regarding the search for life outside of Earth since the discovery of their liquid water. Future spaceflight missions to these moons will likely focus on the search for biosignatures as possible evidence of life. Specific patterns of organics could serve as one line of evidence for the presence of extant or past life as detailed in Creamer *et al*. To achieve this goal, it is necessary to develop spaceflight-compatible sample preparation and analysis methods for detection of organic substances that could be present in samples collected from the surface or plumes. Capillary electrophoresis (CE) is a relatively simple separation technique that can be used for these studies. In this project, a CE-based method for the separation of small organic compounds isolated from bacteria that could serve as potential biosignatures is described. Both UV and capacitively coupled contactless conductivity detection (C^4D) were employed for the determination of several key metabolites present in bacterial cells, including amino acids, nucleobases/sides, and glutathione. Lysates of *E. coli* and *P. haloplanktis* were prepared and analyzed using the CE-C^4D-UV method. The major metabolites that were detected were oxidized and reduced glutathione and several amino acids. The patterns of metabolites differed between the two species. The ultimate goal of this project is on-line extraction and analysis; therefore, a prototype 3D printed device containing a nylon membrane filter was also developed for cell trapping, subsequent lysis, and metabolite extraction. The effect of the extraction solvent used on the metabolite profile obtained for bacteria samples lysed using the 3D printed device was investigated. The optimized extraction device was able to efficiently trap and lyse cells and several major cell metabolites were identified by CE-C^4D-UV.

Reference: Creamer, J. S., *et al*. *Anal. Chem.* **2017**, *89* (2), 1329-1337.

#19 - Synthetic Modification of Sanctolide A Reactivity

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Due to the hydrolytically unstable nature of the enamide-containing, macrocyclic natural product sanctolide A, little is known about its biological activity. To address this problem, a series of more stable, α,β-unsaturated ester and acrylamide analogs were synthesized to investigate the dependence of thiol-Michael addition kinetics and selectivity on the electrophilic warhead. The synthetic route employs a $AlMe_3$-mediated lactone opening/amide formation step, subsequent esterification to various amino acid-derived subunits, and macrocyclization by ring-closing metathesis. Kinetics of thiol addition to the macrocycles is then accomplished by ^{19}F NMR experiments.

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

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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 ft^2 BSL-2 facility equipped with a BD FACSymphony™ S6 Cell Sorter, a BD FACSAria™ Fusion cell sorter, a Cytek™ Aurora Spectral Flow Cytometer, and other supplemental assay instrumentation. The Cytek™ Aurora full-spectrum flow cytometer 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.

#21 - Comparing the use of S-Trap and DNA Miniprep Columns for the Digestion of an Ovarian Cancer Biomarker

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Enzymatic digestion is a key step in a bottom-up proteomics workflow. With current methods adopting the use of detergents for denaturation and solubilization, a filtration step prior to enzymatic digestion has become a necessity. Inclusion of a filtration step in the proteomics workflow allows for the use of detergents that are protease incompatible and can also be harmful to mass spectrometers. Suspension trapping (S-Trap) has been previously used for this filtration step but with the high cost of individual S-Traps ($9.99 per unit), a new mode was sought out. A prior study from Mousseau and coworkers investigated the use of DNA spin-filters, or “Minipreps,” as a replacement for S-Trap filtration. Minipreps

employ a silica-based filter that allows for protein binding through negatively charged silica and washes away the harmful detergents used during denaturation. Minipreps have shown to be advantageous for bottom-up proteomics due to a lower cost (~$0.45 per unit) when compared to its competitors. In this previous study, Mousseau and coworkers identified the possibility of Minipreps being a suitable replacement for S-Traps through the digestion of *E. coli* cell lysate. Further steps were needed to validate if this preparation method is viable for clinical setting. As mass spectrometry is becoming a popular choice of assay for disease detection, being able to confirm preparation methods are compatible for biologically complex samples, such as human samples, is essential. In this current work, a side-by-side digestion of conditioned cell media from an ovarian cancer cell line, OVCAR3 cells, using both S-Traps and Minipreps is performed. The mass spectrometry data obtained is then analyzed to compare the percent coverage of MUC16, an ovarian cancer biomarker, along with a classification of all proteins identified. Proteins are classified into categories such as isoelectric points and hydrophobicity to acknowledge any bias for or against either preparation method.

#22 - Antibiotic Adaptation Alters Resistance Effects of *Pseudomonas aeruginosa* Quorum Sensing-null Mutants

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In patients with cystic fibrosis, a significant cause of morbidity and mortality is chronic infections of the lung. These infections are often caused by the bacterial pathogen *Pseudomonas aeruginosa*, which can become highly antibiotic resistant over the course of years or even decades. Antibiotic resistance can limit treatment options; thus it is important to understand how antibiotic resistance emerges in these isolates. A common adaptation in clinical *P. aeruginosa* isolates is null mutations in the gene encoding LasR, which is involved in a type of population density dependent gene regulation called quorum sensing. In laboratory experiments the loss of quorum sensing increases sensitivity to the clinically relevant antibiotics ceftazidime and tobramycin, raising the question of how *lasR* mutations can emerge in patients treated with these antibiotics. In prior work, we demonstrated that a specific mutation in the elongation factor gene *fusA1* (G61A) can reverse the effects of LasR on tobramycin resistance, which could potentially explain the emergence of *lasR* mutations in clinical isolates. Interestingly, the same *fusA1* mutation abolishes the effects of LasR on ceftazidime resistance. These results suggest the *fusA1* G61A and *lasR* mutations can cause resistance effects through different pathways. Here, we explore the mechanisms of these effects. We used gene expression analysis to identify genes that were regulated by LasR and *fusA1* G61A mutations in a pattern correlating with the observed antibiotic resistance effects. For ceftazidime, the gene expression patterns correlated with several enzymes involved in detoxification of reactive oxygen species. For tobramycin, we identified *fusA2* encoding an alternate elongation factor gene. We tested the role of *katA* and *fusA2* in modulating resistance effects by expressing them from a synthetic promoter from a neutral site in the genome. Expressing *katA* restored ceftazidime resistance to a *lasR* mutant, and expressing *fusA2* abolished the effects of the *fusA1* mutation in a *lasR* mutant. Together, these results provide mechanistic insights into how *fusA1* and *lasR* mutations interact to modulate antibiotic resistance, and new insight into how genetic mutations can alter the course of evolution under selection by antibiotics.

#23 - Host Glycan Utilization is Influenced by Multiple Transcription Factors in *Enterococcus faecalis*

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Enterococcus faecalis is a gram-positive commensal that colonizes the GI tract of most animals including humans. It is also an opportunistic pathogen and causes serious infections including bacteremia and UTI. Many *E. faecalis* strains also exhibit resistance to many antibiotics. Previous studies demonstrated the ability of *E. faecalis* to utilize host-derived glycans as a carbon source, and several *E. faecalis* glycosyl hydrolases have been implicated in utilizing these carbon sources. We focused on identifying regulatory factors that control expression of proteins involved in glycan uptake and processing. The promoter regions of three genes involved in glycan transport (*ef2223*) and glycan processing by alpha-mannosidases (*ef1708*, *ef2217*) were inserted into a luciferase reporter plasmid (pKS320). *EF2223* is the first gene of a larger operon (*ef2223-21*) that encodes a novel ABC transporter. *EF1708-07* is a predicted two gene operon encoding a GH38 family glycosyl hydrolase (EF1708), predicted to cleave the α-1,6- N-linked mannose residues, and EF1707 is a predicted GH125 family glycosyl hydrolase that cleaves α-1,3- N-linked mannose residues. EF2217 is GH92 family member shown to cleave the α-1,2- N-linked mannose residues. A luciferase assay was conducted to observe the expression of the target genes. These luciferase reporter plasmids were introduced into the parental V583 strain, as well as Δ*ccpA*, Δ*yesN* and Δ*ef1709* strains to assess the role of these various transcription factors on gene expression with various carbon sources. Results of these experiments show that each transcription factor plays a role in regulating host glycan utilization in *E. faecalis*.

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

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Microorganisms living in the human gut have long been known to protect their hosts against pathogens. We previously showed that organic extracts containing small molecules from human feces significantly alter global gene expression in the human pathogen, *Vibrio cholerae.* In particular, the expression of motility and chemotaxis genes was markedly reduced in cultures grown in the presence of the fecal extract. Here, we focus on one gut microbiota species, *Enterocloster citroniae,* and demonstrate that extracts from pure cultures of this commensal can also dampen *Vibrio cholerae* motility. We have also established infection protocols using *V. cholerae* and A-549 and HT-29 cell lines to investigate the impact of *E. citroniae* extracts on *V. cholerae* interactions with host cells. Furthermore, to determine the chemical nature of active compound(s), a series of purification methods culminating with High Performance Liquid Chromatography assays will be adopted. Mass spectrometry and nuclear magnetic resonance (NMR) assays will be performed to determine the chemical composition of active compound(s). To that end, we have used ^{1}H and ^{13}C NMR to profile the chemical composition of extracts from active strains, and common features could be detected. Once compound(s) are purified and the structures are identified, we will investigate the effects of pure compounds on *V. cholerae* behavior. Together with previous studies, this work will shed light on *V. cholerae* interactions with its host and the associated gut microbiota through the lens of chemical biology.

#25 - The Coronavirus Macrodomain Mac1 Counters an Innate Antiviral Mechanism Targeting Early Viral RNA Transcription

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All coronaviruses (CoVs) contain a macrodomain, termed Mac1, in non-structural protein 3 (nsp3) which binds and hydrolyzes ADP-ribose covalently attached to proteins. Previous work has established the role of MHV Mac1 as a key virulence factor that counters antiviral PARP activity. Subsequent comparison between single point mutations abrogating catalytic activity (N1347A) and substrate binding affinity (D1329A) suggested multiple contributions of Mac1 to the overall viral life cycle. Furthermore, a double N1347A/D1329A mutant and an in-frame Mac1 deletion mutant were unrecoverable, demonstrating Mac1 as essential for MHV replication. Interestingly, a Mac1 deletion was also detrimental for MERS-CoV, but not SARS-CoV-2, indicating that some CoVs may have evolved to contain genes with redundant functions with Mac1. To investigate the specific role of Mac1 in the viral life cycle, we used the D1329A MHV mutant which, while replication competent, displayed the most severe growth defects. The D1329A mutant generated fewer and smaller plaques, proliferated more slowly, and produced far fewer infectious progeny than WT MHV. To clarify the source of this defect, we assayed plaque forming units against intracellular viral gRNA levels and determined that viral entry was unaffected by Mac1 mutation. Further qPCR data and confocal microscopy observations confirmed a large defect in the initiation of genomic and subgenomic RNA following D132A infection compared to WT virus. These results indicate that Mac1 counters an intrinsic, ADP-ribosylation dependent, antiviral response. Efforts are ongoing to identify precisely how the host represses D1329A.

#26 - PARP14 both Inhibits and Promotes the Replication of Multiple Viruses

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ADP-ribosylation is known to regulate a variety of cellular processes. This modification is carried out by ADP-ribosyltransferases known as PARPs and catalyze the addition of mono- or poly-ADP-ribose units on to a proteins. Several viruses, including coronavirus, encode a macrodomain (MD) protein which catalyzes the removal of mono-ADP-ribose units from proteins, reversing PARP activity. Using reverse genetics and inhibitor studies, we found that PARP14, a mono-ADP-ribosylating PARP, promoted interferon signaling in A549 cells and Bone-marrow derived macrophages (BMDMs) and restricted the replication of the MD mutant murine coronavirus MHV-JHM in macrophages and dentritic cells, suggesting the involvement of PARP14-dependant ADP-ribosylation being a potent antiviral factor during coronavirus replication. Interestingly, we also observed that PARP14 inhibited the replication of a negative sense single stranded RNA genome containing lymphocytic choriomeningitis virus (LCMV) and a DNA genome containing Herpes Simplex Virus-1 (HSV-1). Counter-intuitively, PARP14 promoted the replication of viruses with different types of negative sense single stranded RNA genome like Ebola, Nipah virus and VSV suggesting that PARP14 influences the replication of different viruses. Given the effect of PARP14 on the replication of viruses belonging to different families, we hypothesize that PARP14 plays an important role in modulating pathways, which affects the replication of these viruses. Hence, future studies will entail identifying the molecular pathway interactions by which PARP14 affects the replication of these viruses.

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

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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.

#28 - Role of Specific CDKs in HSV-1 Lytic Infection

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Herpes simplex virus 1 (HSV-1) is a DNA-containing enveloped virus that can cause oral, facial, or genital sores. In certain instances, viral infection results in keratitis and life-threatening encephalitis. Similar to other herpesviruses, HSV-1 has two phases of infection: lytic and latent. One viral protein that regulates the HSV-1 lytic-latent cycle is infected cell protein 0 (ICP0). ICP0 is a phospho-protein that stimulates viral gene expression and replication and is required for efficient reactivation from latency. Notably, ICP0's transactivation activity and its ability to impair the host's antiviral responses require its E3-ubiquitin (Ub) ligase activity. Because HSV-1 is an obligate intracellular pathogen, cellular factors are needed for its replication. Published studies and a high-throughput assay developed in our laboratory indicate the activities of several cellular cyclin-dependent kinases (CDKs) are required for HSV-1 replication and ICP0's transactivator function. To determine which CDKs regulate HSV-1 and ICP0, we tested specific inhibitors of CDK-1, CDK-2, and CDKs-4/6 in viral yield assays in a human cell line using a cycloheximide block and release protocol. We observed that inhibitors of CDK-1 and -2 impaired wild-type virus (ICP0+) replication, whereas an ICP0-null mutant (ICP0-) was impaired by the CDK-2 inhibitor. These results strongly suggest that CDK-1 activity significantly enhances viral replication in an ICP0-dependent manner, whereas CDK-2 appears to act in a predominantly ICP0-independent manner. Future studies will examine how specific CDK inhibitors alter the activities of ICP0 and the HSV-1 lytic-latent cycle.

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

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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.

#30 - Chronic Treatment Tamoxifen Increases Dopamine Release and Inhibits Dopamine Reuptake

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Dopamine is an important neurotransmitter responsible for locomotion, the regulation of mood and more. Dysregulation of dopaminergic systems in the mesolimbic pathway, which is commonly associated with mediating rewards, is implicated in disorders such as depression, Alzheimer's disease, Parkinson's disease, and Post-Chemotherapy Cognitive Impairment (PCCI). Previously our lab has shown that chemotherapies alter dopamine release in a drug dependent manner. Tamoxifen is chemotherapy belonging to a class of drugs called selective estrogen receptor modulators, however the effects of chronic tamoxifen and hormonal treatments on the dopaminergic systems have not been fully elucidated. Despite a lack of research on tamoxifen, patient reports of cognitive impairments with tamoxifen last longer than in patients who do not take tamoxifen. A previous study from the Ewing lab has shown quantal release of dopamine from PC12 cells to be reduced when bath treated with tamoxifen, but little research has been done in tissues. Using fast scan cyclic voltammetry, this work shows that chronic treatment with tamoxifen increases dopamine release in the striatum while decreasing the kinetic rate of reuptake for dopamine. These alterations may increase activation of post-synaptic neurons and play a role in the pathology of PCCI.

#31 - Study of Peptides to Modulate Autoimmunity

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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] Autoimmune diseases are conditions that result from an anomalous response of the immune system, wherein it mistakenly targets and attacks healthy, functional parts of the body as if they are foreign organisms.[2] 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 and cytotoxicity across various cell lines.

Methods: Mouse bone-marrow derived dendritic cells (BMDCs) were harvested from C57BL/6 mice. In BMDC activation assays, cells were plated and treated with various concentrations (10 uM, 1 uM and 100

nM) of five peptides ($OVA_{323-339}$, $OVA_{257-264}$, LABL, $OVA_{323-339}$BPI, $OVA_{257-264}$BPI), using imiquimod as a positive control for immune stimulation. After 48 h incubation, cells were harvested, and supernatants were collected. The cells were then washed and stained with fluorescently-labeled mAbs to measure costimulatory molecule expression (CD40, CD80, CD86) by flow cytometry. Enzyme-linked immunosorbent assays (ELISA) kits were used to examine the expression of IL-10 and TNF-alpha. CaCo-2 cells were used when running a lactate dehydrogenase (LDH) cytotoxicity assay with the same concentrations and treatment groups.

Results: When evaluating the BMDC cell markers there was no significant difference in expression levels between the different treatment groups. Evaluating the ELISA IL-10 and TNF-alpha data showed no significance in upregulation of these cytokines. LDH assay data demonstrated that peptides have negligible cytotoxicity against the CaCo-2 cells.

Conclusions: Continued investigation is needed to discover more potent therapeutics with targeted immunomodulation. We will continue with these concentrations going further as we see there is no cytotoxicity at our highest concentration of 10 uM. Ongoing testing will continue to employ immunophenotyping through flow cytometry as well as cytokine quantification via ELISA. Further studies are needed to identify other cell markers as well as cytokines when immune cells are exposed to these peptides. Finally, in-vivo disease model studies will evaluate these treatments as potential therapeutic regimes.

References: 1. NIH Autoimmune Diseases Coordinating Committee: Autoimmune Diseases Research Plan, March 2005, https://www.niaid.nih.gov/sites/default/files/adccfinal.pdf 2. Goldmuntz, E., et al. “Autoimmune diseases". Office on Women’s Health. U.S. Department of Health and Human Services. February 22, 2021. https://www.womenshealth.gov/a-z-topics/autoimmune-diseases

#32 - Thyroid Hormone Action in Mechanisms of Myelin Repair

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Thyroid hormone is established to play an important developmental role in the central nervous system, but it also plays important role in central nervous system repair. We are particularly interested in the role of thyroid hormone in microglia. Thyroid hormone induces phagocytosis in microglia by activating TREM2. TREM2 is a cell surface receptor on macrophages and microglia that triggers these cells away from a pro-inflammatory state to an anti-inflammatory, phagocytotic, restorative phenotype. Ultimately, microglia will phagocytose myelin debris during disease which is necessary to initiate proper myelin repair. In addition, the literature has recently indicated that TREM2-deficient microglia phagocytose myelin debris, but fail to metabolize cholesterol, resulting in the accumulation of cholesterol esters. Interestingly, the Hartley lab has also shown that there are elevated levels of cholesterol esters in the brains of demyelinated mice, suggesting that cholesterol is not being appreciably metabolized. We have also shown that treatment with a thyroid hormone agonist, Sob-AM2, which is known to promote remyelination, reduces the accumulation of cholesterol esters during demyelination. Therefore, we plan to develop a microglia-based assay to investigate the exact mechanism of cholesterol ester metabolism in microglia. Understanding how thyroid hormone affects the mechanisms of myelin lipid debris clearance will enable the development of better treatments for demyelinating diseases.