# 2025 Molecular Biosciences Symposium

The symposium is hosted by the 4th year graduate students and sponsored by:

K-INBRE

KU CHEMICAL BIOLOGY OF INFECTIOUS DISEASE
The University of Kansas
Center of Biomedical Research Excellence

MB GSO
Molecular Biosciences Graduate Student Organization

The University of Kansas Cancer Center

# 2025 Molecular Biosciences Departmental Symposium

Friday, August 15th, 2025
Location: Burge Union Forum CD

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|---|---|
| **Opening Activities** | |
| 8:30 AM | Poster set-up & refreshments |
| 9:00 AM | Welcome |
| **Faculty Talks** | |
| 9:10 AM | Dr. Caetano Antunes<br>*Antivirulence activity of the human gut microbiome* |
| 9:40 AM | Dr. Coral Zhou<br>*Mechanisms of Genome Scaling in Frogs* |
| 10:10 AM | Dr. Emily Beck<br>*Lies, Damned Lies, and Mitochondria* |
| 10:40 AM | Break |
| **Keynote Speaker** | |
| 11:00 AM | Dr. Heba Mostafa, Johns Hopkins School of Medicine<br>*Genomic Virus Evolution: Impact on Disease Incidence and Outcomes* |
| 12:00 PM | Lunch |
| **Student Talks** | |
| 1:00 PM | Evan Schulz<br>*Role of Ewing sarcoma proteins in the mitotic spindle* |
| 1:20 PM | Vedant Jain<br>*VAB-8/KIF26, EFN-4/Ephrin, and LIN-17/Frizzled control distinct phases of Q cell migration* |
| 1:40 PM | Anam Shaikh<br>*PEP-R619W Distinctly Modulates Persistent Type-I Interferon Signaling to enhance DC and CD8 T cell function* |
| 2:00 PM | Andrew Daufel<br>*Catanionic Detergent Vesicles: A Novel Outer Membrane Biomimetic* |
| 2:20 PM | Break |
| **Poster Sessions** | |
| 2:30 – 3:15 PM | Poster Session I |
| 3:15 – 4:00 PM | Poster Session II |
| **Picnic & Social** | |
| 5:30 PM | Picnic & Social at Holcom Park |

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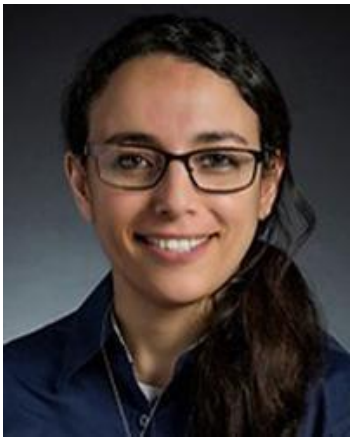
Keynote Speaker

# Genomic Virus Evolution: Impact on Disease Incidence and Outcomes




**Dr. Heba Mostafa Ph.D.**,

Director, Molecular Virology Laboratory, The Johns Hopkins Hospital
Associate Professor of Pathology, Johns Hopkins School of Medicine


The complexity of laboratory diagnostic algorithms for respiratory pathogens hinders a clear understanding of the association between infrequently screened viruses, symptomatic infections, and disease severity. The absence of rapid diagnostic approaches for novel pathogens further delays early diagnosis and outbreak containment. The COVID-19 pandemic underscored critical gaps in diagnostics, surveillance, and early detection protocols.

To address these gaps, it is essential to establish a robust infrastructure for the surveillance of respiratory pathogens. While genomic surveillance of SARS-CoV-2—and to a lesser extent, influenza—is a nationally coordinated effort, surveillance of other clinically significant respiratory viruses remains limited. For example, adenovirus has recently been implicated in cases of acute hepatitis in children, and Enterovirus D68 was associated with severe illness and increased ICU admissions across the U.S. in 2022. Despite their clinical impact, both virus groups lack structured surveillance systems, and data linking viral genomic changes to clinical outcomes are scarce. Additionally, the effects of newly recommended RSV vaccines on viral evolution remain unknown. Collectively, these factors highlight the urgent need to systematically analyze the clinical outcomes associated with the most prevalent respiratory viruses and to understand how circulating genotypes and viral evolution contribute to disease severity and outbreak dynamics. The goal of our research is to develop an efficient genomic surveillance framework to track respiratory virus evolution and identify genomic features linked to severe disease or emerging outbreaks.

Faculty Talks

# Antivirulence Activity of the Human Gut Microbiome

**Caetano Antunes**
Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045

Our group studies the role of small molecules in host-microbiota-pathogen interactions. We have thus far focused on the impact of microbiome-derived compounds on enteric pathogen behavior. Using untargeted metabolomics, we previously showed that the human gut harbors thousands of small molecules, most of which are unknown. The gut microbiome is involved in the production of the majority of these compounds, as microbiome disruption through antibiotic treatment resulted in altered levels of more than 85% of the metabolites detected. We then hypothesized that some of these compounds may elicit responses in various cell types in the gut. Organic extracts of human feces were then used to determine transcriptional responses of pathogens to the chemical milieu of the human gut through mRNA sequencing. Our results showed that multiple enteric pathogens, such as *Vibrio cholerae*, *Salmonella enterica*, and *Clostridioides difficile* display marked transcriptional responses to the human gut metabolome, and that genes required for host interactions and virulence are modulated. In *V. cholerae*, we showed that swimming motility is drastically repressed in the presence of fecal extracts. Also, we were able to isolate and identify bioactive members of the gut microbiome. Pure cultures of various species of *Enterocloster* produce bioactive compounds that repress *V. cholerae* swimming motility, recapitulating the effect of the fecal extract. Due to the repressive effect on motility, we predicted that biofilm formation would also be affected by bioactive commensals. Indeed, *V. cholerae* produced significantly more robust biofilms in the presence of *Enterocloster*-derived compounds. Host cell interaction assays using cultured colonic epithelial cells showed that small molecules produced by *Enterocloster* also modulate toxin-mediated host cell death induced by *V. cholerae*. Previously, we have been able to identify microbiome-derived bioactive compounds that affect *S. enterica* virulence, the most active of which was 3,4-dimethylbenzoic acid (DMB). Interestingly, DMB is not responsible for the effect on *V. cholerae*, suggesting that a new bioactive compound is involved. Ongoing work is focused on identifying the bioactive compound produced by *Enterocloster* and revealing the molecular mechanisms behind bioactivity against *V. cholerae* and *S. enterica*.

# Mechanisms of Genome Scaling in Frogs

**Coral Zhou**
Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045

Across the tree of life, genome size varies by six orders of magnitude. Polyploidy is an extreme form of genome size expansion in which entire copies of the genome are duplicated. During evolution, polyploidy correlates with bursts of speciation and genetic innovation. Within a species, including in humans, polyploid cells have specialized functions in many tissues including the liver, eye and reproductive organs. Across this huge range, the ratio between genome size, nuclear size, and cell size is largely conserved for a given species and cell type. Deviations in nuclear-to-cytoplasmic (N/C) ratio are strongly correlated with pathologies such as aging and cancer through unknown mechanisms. In the Zhou Lab, we use naturally polyploid African clawed frogs Xenopus to discover the basic molecular mechanisms that sense and adapt to changes in genome size during embryogenesis and evolution. Gaining this knowledge is critical for understanding how genomes maintain homeostasis while creating new functions during health and disease.

# Lies, Damned Lies, and Mitochondria

**Emily Beck**
Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045

"Mitochondrial diseases" is a prevalent group of diseases characterized in part by mitochondrial (mito) dysfunction and includes Parkinson's disease, Alzheimer's disease, multiple sclerosis, cancer, diabetes, and more. Mito-dysfunction leads to a wide range of symptoms because mitochondria play many roles in regulation of cellular health including energy production and regulation of the cell cycle, cell death, calcium homeostasis, lipid biosynthesis, and aspects of the immune response. Mitochondria also contain their own genomes (mitogenomes) encoding products that must physically and functionally interact with nuclear-encoded products to maintain function. There are two conflicting views on mitogenomes that have major implications for how we approach understanding the genetic basis of mito-dysfunction. (1) They are a genetically stable and reliable population genetic marker and (2) they are a harbor of genetic variation. Understanding the genetic basis of mito-dysfunction has been a challenge. We know genomic variation between individuals plays a strong role in etiology of disease, but unlike the nuclear genome, we cannot edit the mitogenome ubiquitously and tools for understanding mitogenomic variation are limited. Recent advances in long-read sequencing technology have expanded our understanding of mitogenomic structure and revealed that mitochondrial heteroplasmy – the presence of more than one mitogenomic type within an individual – is more common than previously thought. Heteroplasmy is particularly relevant to our understanding of mito-dysfunction as symptoms often only occur at a "mitochondrial threshold effect" where one haplotype reaches a particular threshold within a cell or tissue. My lab works on a suite of organisms that have evolved unique mitogenomic features that allow us to understand the role of mitogenomic variation in maintaining mito-nuclear genomic interactions. Our recent work is challenging what we thought was possible of mitogenomic variation. Today I will discuss these new data for the first time.

# Student Talks

## Role of Ewing Sarcoma Proteins in the Mitotic Spindle

**Evan Schulz[1],** Mizuki Azuma[1]
[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS

Ewing sarcoma (ES) is an aggressive pediatric bone tumor in critical need of more effective and targeted chemotherapies with fewer side effects. ES's driving hallmark, the t(11:22) chromosomal translocation, generates two aberrations of the EWS protein: the loss of an *EWS* allele and expression of *EWS/FLI1* fusion protein, denoted as EWS aberrations. Identifying drug targetable EWS aberration-induced changes to tumor biology and drug sensitivity is required to optimize current treatment strategies and develop new targeted therapies. Our previous work found that EWS aberrations can defect chromosome segregation in mitosis. **In this project, we hypothesized that EWS and EWS/FLI1 localize to the mitotic spindle, with EWS aberrations defecting mitotic spindle structure and increasing consequent microtubule breaks**. To test this hypothesis, we established a DLD-1 colorectal cancer line that enables conditional exogenous *EWS/FLI1 expression* with doxycycline (Tet-On system), single-allele *EWS knockdown* with auxin (auxin-inducible degron system), or a combination of both (*EWS/FLI1-EWS KD*). Immunocytochemistry revealed that EWS and EWS/FLI1 localize to the mitotic spindle. Upon EWS knockdown or EWS/FLI1 expression, mitotic spindle displayed strikingly abnormal geometries and fiber defects. Since defects in the mitotic spindle or fiber structure can result in microtubule lattice damage, we probed cells with an antibody known as MB11 that visualizes microtubule damage and repair sites. This assay demonstrated that EWS knockdown and EWS/FLI1 expression increase spindle microtubule damage, with an additive effect in combination. Further, as microtubule lattice damage sites are key structural vulnerabilities to microtubule destabilizing drugs (MtDs), we tested whether EWS aberrations sensitize cells to microtubule disruption by nocodazole. This assay showed that EWS knockdown and EWS/FLI1 expression sensitize cells to nocodazole in combination. Taken together, our data support our hypothesis and suggest that the combination of *EWS/FLI1-EWS Knockdown* increases nocodazole sensitivity by inducing spindle structural defects and microtubule damage sites. In parallel with these experiments, we compared ES cell lines and mesenchymal stem cells (ES cell of origin), finding that ES spindles share striking structural similarities with spindles subjected to EWS knockdown or EWS/FLI1 expression, also contain high levels of spindle microtubule damage, and are far more sensitive to nocodazole than MSCs. We are currently investigating the mechanism of EWS/FLI1-induced mitotic spindle defects and damage, and the role of EWS/FLI1 expression in MtD chemosensitivity.

# VAB-8/KIF26, EFN-4/Ephrin, and LIN-17/Frizzled Control Distinct Phases of Q Cell Migration

**Vedant Jain[1],** Erik Lundquist[1]
[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS

Q cell neuroblasts are a pair of bilateral neuroblasts that are born in the posterior-lateral region of the animal *C. elegans*, with QL and QR on the left and right, respectively. Initially, QR migrates anteriorly over the V4 seam cell, whereas QL migrates posteriorly over the V5 seam cell. The second phase of migration is Wnt-dependent and begins after this initial phase. QL descendants QL.a/p encounter EGL-20/Wnt, a posteriorly expressed Wnt ligand, leading to the initiation of the canonical Wnt pathway and expression of the MAB-5/Hox transcription factor in the daughter cells. *mab-5* expression in QL.a enables its migration posteriorly over QL.p, after which QL.a undergoes cell division to generate two daughter cells QL.aa and QL.ap. QL.aa undergoes apoptosis, and QL.ap continues migration posteriorly and differentiates into the PQR neuron. *MAB-5/Hox* is both necessary and sufficient for this posterior migration process, as its ectopic expression in QR results in posterior migration of QR.ap (AQR neuron). We utilized FACS sorting and RNA-seq of early L1 larval Q cells to identify and discover genes that are differentially regulated by *mab-5* in *lof* and *gof* alleles. In addition to discovering the genes that act downstream of MAB-5, we have defined three distinct stages of neuroblast migration using *in vivo* imaging. Our results suggest that VAB-8/KIF26, LIN-17/Frizzled, and EFN-4/ephrin play a necessary role in distinct phases of QL cell migration. VAB-8/KIF26 plays a role in the first, second, and third stage of migration, LIN-17/Frizzled plays a necessary role in the second and third, whereas EFN-4/Ephrin only plays a role in the third stage of migration. Overall, this approach has led to a comprehensive understanding of how a Hox gene regulates distinct phases of neuroblast migration *in vivo*.

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

**Anam F. Shaikh**[1], Jenna Barnes, Nancy Schwarting, Tammy Cockerham, and Robin C. Orozco[1]
[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS

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

# Catanionic Detergent Vesicles: A Novel Outer Membrane Biomimetic

**Andrew Daufel**[1]

[1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS

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

# Poster Session Roster

## Poster Session I (2:30-3:15 PM): Even number posters

## Poster Session II (3:15-4:00 PM): Odd number posters

| No. | Presenter | Title |
|---|---|---|
| 1 | Kervens Accilien | Differential Evolution of *Pseudomonas fluorescens* against Ribosome-Targeting Peptide vs Small-Molecule Antimicrobials |
| 2 | Tolulope Ade | Transcription and Regulation of the mpt PTS in *Enterococcus faecalis* |
| 3 | Taiye Adewumi | Adaptation of a DNA virus to *Drosophila melanogaster* |
| 4 | Syeda Kishuara Begum | Inflammation and Epigenetic Priming in Zebrafish Induced by Early-Life Hypoxia: A Developmental Basis for Cardiovascular Disease |
| 5 | Alec Bevis | The Autoimmunity-Associated Minor Allele of PTPN22 Enhances Innate Antiviral Immunity During Coronavirus Infection |
| 6 | Alfred Buabeng | Targeting HuR-Mediated Regulation of CD147+Extracellular Vesicles to Modulate Immune Responses in Triple-Negative Breast Cancer (TNBC) |
| 7 | Asbin Chand | Haspin Kinase Regulates a Topoisomerase II-Dependent Metaphase Checkpoint Critical for Chromosome Segregation |
| 8 | Larissa Da Silva Ferreira | The Human Gut Metabolome Alters *Vibrio cholerae* Behavior |
| 9 | Ryan Grigsby | University of Kansas Nanofabrication Facility: Equipment and Services |
| 10 | Daniel Montezano | Exploring Uncharted Protein Sequence Space in de novo Barrel Generation |
| 11 | Moises Gualapuro | Structural and functional prediction of the hypothetical *Chlamydia trachomatis* proteome |
| 12 | Bhavya Gupta | Structural Determination of the ColE1* and TolC Complex |
| 13 | Jennifer Hackett | Next Generation Sequencing at KU Genome Sequencing Core |
| 14 | Kiana Hajiarbabi | Commensal *Staphylococcus epidermidis* produces compounds that inhibit *Staphylococcus aureus* pathogenesis |
| 15 | Priyani Hapugaswatta | Aneuploidy induction mechanism in Ewing sarcoma |
| 16 | Maya Hull | Knockdown of ER Quality Control Rescues Drosophila Model of the Rare Disease DPAGT1-CDG |
| 17 | Patryk Hupert | Bioanalytical Investigation of Metabolic and Transcriptomic Profiles in a Human Tri-Culture Neuroinflammation Model |
| 18 | Sumaya Afnan Jahin | Genetic adaptation alters the antibiotic susceptibility of quorum sensing (LasR)-null mutants in *Pseudomonas aeruginosa* |
| 19 | Anika James | Nuclear APC maintains colon homeostasis and mitigates inflammation |
| 20 | Andrew Johannesen | Ultra-High-Throughput Virtual Screening & Binding Site Prediction |
| 21 | David Johnson | The Computational Chemical Biology Core: A core lab |
| 22 | Nicasio Jurani | Initial characterization of post-embryonic gut development in the ctenophore, *Mnemiopsis leidyi* |
| 23 | Sunghae Kim | Inhibition of HuR in Cancer Cells Enhances T Cell Activation and Sensitizes Tumors to Immunotherapy |
| 24 | Kate Kirby | Gut Commensal Metabolites Modulate *V. cholerae* Biofilm Growth |

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|---|---|---|
| 25 | Katherine Lenz | Mechanisms of Genome Scaling in Frogs |
| 26 | Yan Luo | Intravenous Recombinant Human ApoE2 Protein Sex-Specifically Improves Serum Lipidomic Profile in Human ApoE4 Knock-In Mice |
| 27 | Peter McDonald | Flow Cytometry Core: A Chemical Biology of Infectious Disease COBRE Core Laboratory |
| 28 | Peter McDonald | University of Kansas Shared Resource Laboratories (SRLs) and Biomedical Research Cores |
| 29 | Mark Yorio | The Hox gene *mab-5* controls left-right asymmetric development of the nervous system in *Caenorhabditis elegans* |
| 30 | Abdulrahman Naeem | The ABC transporter EF2223-EF2221 of *Enterococcus faecalis* imports high mannose glycans, and is dependent on a three-component signal transduction system |
| 31 | Saeideh Nasiri | Gut microbiome-derived metabolites modulate *Vibrio cholerae* interactions with host cells |
| 32 | Noshin Nawar | Mitochondria, Microbes and the Aging Mind |
| 33 | Vanessa Nguyen | Clusterin (CLU) as a Modulator of Neuronal Excitatory/Inhibitory Balance |
| 34 | Ngoc Nguyen | Up-Regulating the cGAS-STING Pathway via HuR Inhibition to Enhance Immunotherapy in Prostate Cancer |
| 35 | Zach Opoka | Assessing the HIF pathway in Ctenophores |
| 36 | Candice Osagie | Investigating the Role of the RNA-Binding Protein HuR in Pancreatic Cancer |
| 37 | Gabby Perkins | Insights into How Chaperone Net Charge and Pattering Influence Nucleic Acid Folding |
| 38 | Jessica Pfannenstiel | Identification of a series of pyrrolo-pyrimidine based SARS-CoV-2 Mac1 inhibitors that repress coronavirus replication |
| 39 | Bikash Pokhrel | RNA binding protein Musashi1 regulates mTOR pathway, potentially controlling growth of the intestinal epithelium. |
| 40 | Macie Proctor-Roser | PTPN22 Impacts the B Cell Landscape During Chronic Virus Infection |
| 41 | Ethan Rogers | Riboregulatory sequence 5BSL3.2 modulates the conformations of 3′X RNA in hepatitis C virus |
| 42 | Anuradha Roy | Infectious Disease Assay Development Core: High Throughput Screening Laboratory at the University of Kansas |
| 43 | Nilanjan Roy | Fly Viral Atlas: Cellular tropism of Drosophila RNA viruses and their impact on the host |
| 44 | Elle Saenjamsai | PARP14 in Host-Virus Interaction: Identifying Domain(s) Required for Viral Replication |
| 45 | Ahnaf Tahmid Saqif | Elucidating quorum sensing-dependent crosstalk in a Burkholderia-Pseudomonas coinfection model |
| 46 | Vanessa Schmidt | Interactions between the *Pseudomonas aeruginosa* Las quorum-sensing system and stringent response reveal distinct mechanisms of resistance to ceftazidime and piperacillin |
| 47 | Azeem Talabi | Molecular Mechanisms of Resistance to Topoisomerase-Targeting Drugs in Adenomatous Polyposis Coli-Mutant Colorectal Cancer |
| 48 | Xiaoqing Wu | University of Kansas – Preclinical Core Facilities |
| 49 | Pranali Yadav | Defining the impact of ApoE isoforms on APP metabolism in humanized Abeta mouse models |
| 50 | Eryk Yarkosky | AHL-specific global gene regulation by a promiscuous receptor in *Chromobacterium subtsugae* |

# A Special Thanks to Our Sponsors

The goal of the CBID is to provide the necessary mentor support and infrastructures to ensure the success of junior investigators and to create a center that encourages basic research scientists to discover Chemical Biology of Infectious Disease. CBID: P20GM113117

KU CHEMICAL BIOLOGY OF INFECTIOUS DISEASE
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THE UNIVERSITY OF KANSAS CANCER CENTER

The University of Kansas Cancer Center is on an urgent journey to break the devastating grip of cancer. We are the only National Cancer Institute-designated comprehensive cancer center in the region, and 1 of only 53 in the nation, to receive this elite distinction. Comprehensive designation is the highest level of recognition awarded by the NCI. It is the gold standard of excellence, awarded only to cancer centers with the deepest and broadest knowledge of cancer.

The purpose of the Kansas INBRE (K-INBRE) is to promote multidisciplinary research networks with a focus on Cell and Developmental Biology increase the research base and capacity through research support; provide a range of basic science and clinical research opportunities for student trainees; serve as a pipeline for students to continue in health research careers in IDeA states; and enhance science and technology knowledge of the state's workforce. K-INBRE: P20 GM103418

K·INBRE
Kansas IDeA Network of Biomedical Research Excellence

MB GSO
Molecular Biosciences Graduate Student Organization

Our organization strives to enrich the lives of graduate students by providing a sense of community through social activities, opportunities to learn about a broad range of science careers and promoting opportunities for graduate students to share their love of science with members of the community.

# Poster Abstracts

1. Differential Evolution of *Pseudomonas fluorescens* against Ribosome-Targeting Peptide vs Small-Molecule Antimicrobials **Kervens Accilien**

   Antibiotics are a powerful tool used to mitigate the effects of pathogen infections on the health and quality of life of human populations. By inhibiting key bacterial function machinery, they ultimately lead to bacterial growth inhibition or death. However, microbes inevitably become resistant to the antimicrobial(s) to which they are exposed. The development of new antibiotics does little to alleviate this problem; once a microbe is exposed to an antimicrobial, resistance follows shortly thereafter. A possible solution to this issue is the development of new classes of antimicrobials against which resistance evolution is harder to achieve. Preliminary studies indicate that peptide antibiotics fit that bill. In a long-term experimental evolution of a microbe, the upper threshold of resistance was several-fold lower for peptides than small molecules. Here, we further explore this phenomenon by examining the differences between peptide-resistant and single-molecule-resistant bacteria. To that end, we selected a set of antimicrobial peptides and small-molecule antibiotics targeting the same biological function (bacterial peptide synthesis). We started by generating a kill curve to determine the minimum inhibitory concentration of each antimicrobial against *Pseudomonas fluorescens*. Next, we generated antimicrobial-resistant populations through experimental evolution. These populations were sequenced and compared to the ancestral population to identify putative resistance-causing mutations. Comparing the mutations identified between populations resistant to the two classes of antimicrobials tells us about the similarities/differences in how our microbe adapts to them. While there was a significant overlap in the SNPs identified in AMP-resistant populations compared to the small-molecule antibiotic-resistant populations there were also some clear differences. Those differences merit further exploration as to how they might be leveraged to target multidrug-resistant microbes.

2. Transcription and Regulation of the mpt PTS in *Enterococcus faecalis* **Tolulope Ade**

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

3. Adaptation of a DNA virus to *Drosophila melanogaster* **Taiye Adewumi**

   Drosophila innubila nudivirus (DiNV) is a double-stranded DNA virus that exhibits high virulence in members of the quinaria group. While much is known about DNA virus adaptation in invertebrates,

questions remain regarding their host range, virulence, and genetic evolution. Here, we explore DiNV adaptation to a D. melanogaster cell line (S2 cells). We injected (thoracic) *Drosophila melanogaster* flies with DiNV into either wild type or Myd88/IMD null mutants and monitored survival for 20 days. Additionally, viral DNA was extracted from both an ancestral DiNV passage from a *Drosophila innubila* cell line and an evolved passage in the S2 cell line (11 passages). Using short-read sequencing we compared genetic differences/mutation between evolved and ancestral DiNV strains. Our results indicate that the virus passaged in S2 cells (evolved strain) demonstrates enhanced growth compared to the ancestral strain. Additionally, we show that the evolved virus exhibits genotype-specific mortality in D. melanogaster, with sequencing identifying potentially causative SNPs. The adaptation of DiNV to S2 cells highlights the significance of viral evolution, demonstrating that DNA viruses in invertebrates can develop host-specific adaptations that affect virulence, this provides insights into DNA virus-host coevolution.

4. Inflammation and Epigenetic Priming in Zebrafish Induced by Early-Life Hypoxia: A Developmental Basis for Cardiovascular Disease **Syeda Kishuara Begum**

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

5. The Autoimmunity-Associated Minor Allele of PTPN22 Enhances Innate Antiviral Immunity During Coronavirus Infection **Alec Bevis**

   Allelic variation can impact the outcome of viral disease. Mice expressing the autoimmunity-associated allelic variant of Ptpn22 (PEP-R619W) can clear chronic LCMV-cl13 infection whereas PEP-WT mice cannot, but little is known regarding its impact during other virus infections. This research defines how the loss of Ptpn22 (PEP-null) and PEP-R619W changes antiviral immunity during coronavirus infection. We addressed the hypothesis that CRISPR/Cas9-generated PEP-null and PEP-R619W mice have enhanced antiviral immunity over PEP-WT mice during coronavirus infection. Following Mouse Hepatitis Virus (MHV) A59 infection, we interrogated pathology, cytokine production, and cellular responses in the spleen, blood, and liver of PEP-WT, PEP-null, and PEP-R619W mice. Key findings show PEP-null and PEP-R619W mice have: 1) reduced weight loss and viral titer, 2) increased survival, and 3) more NK cells in the liver and spleen. Further, Rag1-/- PEP-null and Rag1-/- PEP-R619W mice had increased survival and reduced viral titer over PEP-WT mice. This study is one of the first to investigate the role of Ptpn22 within NK cells. Furthermore, our results demonstrate that this allelic variant of Ptpn22 is beneficial during coronavirus infection.

6. Targeting HuR-Mediated Regulation of CD147+Extracellular Vesicles to Modulate Immune Responses in Triple-Negative Breast Cancer (TNBC) **Alfred Buabeng**

Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer lacking estrogen, progesterone, and HER2 receptors, resulting in limited targeted treatment options and poor survival rates. Emerging evidence suggests that extracellular vesicles (EVs) play a crucial role in TNBC progression and immune modulation. The RNA-binding protein HuR (encoded by ELAVL1) is overexpressed in TNBC and stabilizes oncogenic mRNAs, contributing to tumor growth and treatment resistance. One of HuR's downstream targets, CD147 (EMMPRIN), is a transmembrane protein found on EVs that drives cancer hallmark processes including cellular invasion, migration, immune evasion and treatment resistance. HuR binds to the 3′-UTR of CD147 mRNA, enhancing its stability and protein expression. CD147 is highly expressed in various cancers including TNBCs, and the expression levels correlate with tumor progression and invasion by inducing the production of matrix metalloproteinases (MMPs), degrading extracellular matrix and promoting EV release. Our preliminary data shows that HuR knockout reduces exosome secretion in MDA-MB-231 cells and subsequently reduced CD147 proteins expressed on EVs. We identified small molecule inhibitors (KH compounds) that disrupt HuR-mRNA interactions. Treatment of wild type TNBC cells with the KH compound reduced CD147 proteins on the cell line. However, the combination of immune checkpoint inhibitors and HuR inhibitors have been shown to synergistically enhance T cell activation and sensitization of TNBC tumors to immunotherapy. Based on these findings, we hypothesize that HuR regulates CD147 expression on EVs and targeting HuR-dependent CD17+EVs signaling may improve immunotherapeutic responses in TNBC.

7. Haspin Kinase Regulates a Topoisomerase II-Dependent Metaphase Checkpoint Critical for Chromosome Segregation **Asbin Chand**

   Haspin is a mitotic kinase critical for cellular processes such as chromosome cohesion maintenance, chromosome segregation, and checkpoint activation. During mitosis, Haspin phosphorylates Histone H3 at threonine 3 (H3T3p), creating a docking site for the chromosomal passenger complex (CPC), which includes Aurora B, a key kinase controlling mitotic progression. Numerous studies demonstrated that Haspin overexpression is associated with high-grade tumors and poor prognosis in multiple cancers, yet the mitosis-specific role of H3T3p in cancer cells remains poorly understood. Previous studies showed that the catalytic inhibitor of DNA topoisomerase II (TopoII), ICRF-193, activates a metaphase checkpoint known as the TopoII-Responsive Checkpoint (TRC). We recently found that Haspin inhibition disrupts TRC signaling, leading to premature anaphase onset despite ICRF-193 treatment. This suggests that the Haspin-Aurora B axis is essential for maintaining TRC function. Furthermore, ICRF-193 treatment mislocalizes H3T3p on mitotic chromosomes, likely impairing CPC recruitment and checkpoint fidelity. To investigate this mechanism, we used genome-edited cell lines to dissect Haspin’s role in regulating TRC via H3T3p during mitosis. These results will extend our understanding of Haspin’s involvement in cancers, and targeting Haspin could be a potent therapeutic strategy in cancers with Haspin and TopoII dysregulation

8. The Human Gut Metabolome Alters *Vibrio cholerae* Behavior **Larissa Da Silva Ferreira**

   The human gastrointestinal tract is home to complex and active populations of microorganisms, deemed microbiota. The gut microbiota plays an immense role in manipulating human health and disease; it maintains immune and metabolic homeostasis. Certain microbiota-intrinsic factors, such as interbacterial competition and secretion of antimicrobial substances, can help protect against pathogen colonization in the gut. Nonetheless, altered gut bacterial composition (dysbiosis) has been associated with the pathogenesis of many inflammatory diseases and infections. Our group showed that fecal extracts are able to protect against host cell invasion of *Salmonella enterica* and *Vibrio cholerae* by modulating gene expression. Currently, *Vibrio cholerae* is a significant health concern as it is the causative agent of the diarrheal disease cholera. This work aimed to investigate whether fecal extracts affect the growth and pathogenicity of *V. cholerae*.

Materials and Methods:
This unpaired study compared the growth of *V. cholerae* in the presence of fecal extracts versus an ethyl acetate (EtOAc) solvent control. The same fecal extract was tested at multiple concentrations. Samples consisted of biological triplicates from the same culture plate, using different colonies. Growth was assessed using 18-hour growth curves and colony-forming unit (CFU) counts. This was not a random sample, as samples were derived from a single donor.

A two-sample (unpaired) t-test was performed using a significance level of $\alpha = 0.05$. Descriptive statistics are presented as mean ± standard deviation (SD). A 95% confidence interval (CI) for the difference between means was also calculated.

The mean OD600 for the EtOAc control was 0.2829 (SD = 0.1492, n = 234), and for the fecal extract-treated group it was 0.1291 (SD = 0.0582, n = 234). The t-test yielded a test statistic of 14.69, with 302.31 degrees of freedom and a P-value of $3.08 \times 10^{-37}$, leading us to reject the null hypothesis. The point estimate for the difference between means was 0.1537, with a 95% CI of [0.1332, 0.1743]. These results indicate that fecal extracts significantly inhibit the growth of *V. cholerae*. This supports the hypothesis that gut microbiota components may offer protective effects against enteric pathogens. Biofilm production increased compared to BHI alone, but not significantly when compared to EtOAc. RNA sequencing and qPCR revealed downregulation of key virulence genes such as hapA and tssF. Future directions include improving RNA quality to reduce DNA contamination, testing a second donor for generalizability, and investigating genes more directly related to *V. cholerae* growth.

9. University of Kansas Nanofabrication Facility: Equipment and Services **Ryan Grigsby**

The Kansas University Nanofabrication Facility (KUNF) is a Core Lab supported by the KU Office of Research and the Center for Molecular Analysis of Disease Pathways COBRE. The KUNF primarily caters to researchers who are manufacturing micro- and nanofluidic devices for biomedical research, but has the equipment and resources to accommodate broad research applications with micro- and nanofabrication needs. The core lab consists of about 1,300 ft2 of ISO class 5, 1,700 ft2 of ISO class 6 and 1,250 ft2 of ISO class 7 cleanroom space, housing tools and materials for techniques including photolithography, nano-imprint lithography, plasma (dry) etching (ICP-RIE), wet etching, metal and dielectric material thin film deposition, scanning electron microscopy (VP-SEM), atomic force microscopy, contact angle goniometry, ellipsometry, profilometry, wafer dicing, laser ablation and engraving, 3D printing, hot embossing, and COMSOL software for device modeling. In addition, the facility has numerous microscopes for general inspection, ovens and furnaces, ultrapure water, dedicated process fume hoods and filtered lighting for photolithography.
This facility is under the direction of Dr. Susan Lunte. Services and usage of the facility are available to researchers from all Kansas universities. Training is provided to new investigators and graduate students in the use of micro- and nanofabrication procedures and equipment. In addition, researchers from both non-Kansas academic and private industry institutions may contract with the facility for consultation and services. Hourly and per-use rates apply for facility access, equipment usage, and staff labor. Consultation is free.

10. Exploring Uncharted Protein Sequence Space in de novo Barrel Generation **Daniel Montezano**

Protein sequence space is vast. With twenty options, computers are required for effective sequence space exploration. We have investigated de novo design of full-length sequences of transmembrane β-barrel proteins (TMBB) using long short-term memory (LSTM) models. We assess the ability of a small generative model to explore uncharted protein sequence space within this structural fold. Our training procedure is efficient because of LSTM's low complexity. Comparatively, the number of parameters (161,302 total trainable parameters for 128 nodes per LSTM cell) is ~2% of those used in transformers. We found that the generated proteins computationally resemble the desired barrel fold by correctly capturing statistics of the primary structure and reproducing the β-signal, a C-terminal sequence motif of true TMBBs necessary for insertion in the outer membrane. We also find that synthetic sequences

generated reproduce characteristics of native TMBBs well enough that the designs are readily predicted as TMBBs by three independent predictors that do not directly rely on homology. The sequences have low similarity to known TMBBs. Our results indicate that for the TMBB family it is possible to successfully train low complexity generative models that efficiently explore uncharted sequence space.

11. Structural and functional prediction of the hypothetical *Chlamydia trachomatis* proteome **Moises Gualapuro**

    The functional annotation of the *Chlamydia trachomatis* genome is significantly hampered by its obligate intracellular lifestyle, leaving nearly a third of its proteome designated as "hypothetical." This study bridges a critical knowledge gap by providing a comprehensive structural and functional characterization of 209 of these previously uncharacterized proteins. We employed a state-of-the-art computational pipeline, generating 3D models with the complementary strengths of AlphaFold3 and I-TASSER and subsequently inferring function with DeepFRI, a structure-based deep learning method. Quality assessment of the 209 models revealed a high degree of accuracy; 37 structures (17.7%) showed very high confidence in both local and global conformations, while an additional 110 (52.6%) had high-accuracy global folds and reliable local predictions. These 147 high-quality models served as the foundation for functional annotation via DeepFRI, which links structure to Gene Ontology (GO) terms with a reliability threshold of >0.5. This approach successfully assigned putative biological process terms to 147 proteins, molecular functions to 116, and cellular components to 144, with 112 proteins receiving annotations across all three categories. This comprehensive annotation effort dramatically reduced the portion of the *C. trachomatis* proteome lacking functional data from nearly 33% to just over 10%. A detailed review of these predictions identified potential enzymes, transporters, and effector proteins, offering novel insights into chlamydial intracellular survival and host-pathogen interactions. Our results provide a significant update to the *C. trachomatis* functional map, offering a structurally-validated dataset that immediately opens new avenues for targeted experimental validation and structure-based drug design.

12. Structural Determination of the ColE1* and TolC Complex **Bhavya Gupta**

    Antibiotic resistance is a critical global health challenge, with annual deaths due to infections increasing from 700,000 to 1.29 million in 2019 alone. Efflux pumps, such as the protein TolC in *Escherichia coli*, contribute to resistance mechanisms by exporting antibiotics out of the cell. TolC is the trimeric β-barrel outer membrane protein that interacts with the bacteriocin Colicin E1 (ColE1) and its Shigella sonnei variant ColE1*. Previous studies demonstrate a domain of ColE1 can partially plug TolC, enhancing bacterial susceptibility to antibiotics. Preliminary findings suggest a fragment of ColE1* may additionally plug TolC more effectively than colicin E1. This study aims to produce TolC, incorporate TolC into nanodiscs, and add a peptide fragment of ColE1*. Structural characterization of the TolC-ColE1* complex will be conducted using cryogenic electron microscopy (Cryo-EM). Isothermal titration calorimetry (ITC) data previously obtained indicate trivalent binding of ColE1* to TolC. These findings may contribute to strategies for addressing antibiotic resistance by targeting bacterial efflux mechanisms and efflux plugs more broadly.

13. Next Generation Sequencing at KU Genome Sequencing Core **Jennifer Hackett**

    The Genome Sequencing Core (GSC) is one of three research service core labs in the NIH COBRE Center for Molecular Analysis of Disease Pathways (CMADP) at the University of Kansas (KU). The major mission of the GSC is to provide researchers with next-generation sequencing (NGS) technologies. NGS, carried out in a massively parallel fashion, has been revolutionizing bio-medical research and used in a growing list of applications. Projects supported by the GSC include de novo genome assembly, genome re-sequencing for identification of mutations and polymorphisms, transcriptome analysis (RNA-seq), and epigenomic and gene regulation studies such as ChIP-seq,

Methyl-seq, and small RNA analysis. The GSC enhances the genomics infrastructure already at KU by providing a range of Illumina sequencing platforms including the NextSeq2000 and NextSeq550 (mid-sized genome re-sequencing or transcriptome projects) and the MiSeq (metagenomic or targeted amplicon sequencing projects) to researchers at KU-Lawrence and across the region. To capture the full power of NGS, we provide a range of project support, including project consultation, sample quality check, sequencing library construction, Illumina sequencing, and FASTQ generation and demultiplexing. For latest pricing, current sequencing queue, or other information, visit the Genome Sequencing Core's website: https://gsc.ku.edu/.

14. Commensal *Staphylococcus epidermidis* produces compounds that inhibit *Staphylococcus aureus* pathogenesis **Kiana Hajiarbabi**

    The skin microbiota serves as an essential protective barrier, offering both physical and biochemical defense mechanisms against pathogens. While much of microbiome research has focused on the gut microbiota, the skin's microbial communities remain understudied. Previous studies have shown that cell-free conditioned media (CFCM) derived from commensal *Staphylococcus epidermidis* significantly reduces the biofilm formation of *Staphylococcus aureus*. In this study, we aimed to identify the genetic mechanisms that lead to inhibition and to assess if these compounds affect viability of host cells. Initial experiments confirmed that *S. epidermidis* CFCM significantly reduced *S. aureus* biofilm formation following a 24-hour incubation period. Our data also demonstrates that this inhibitory effect occurs as early as 3 hours post-incubation. Additionally, genetic analysis revealed that biofilm of sarA and srtA transposon mutants were not significantly inhibited by *S. epidermidis* CFCM in comparison with control, suggesting these genes may play important roles in mediating this interaction. Furthermore, when *S. aureus* was grown in the presence of *S. epidermidis* CFCM, we observed a significant reduction in both *S. aureus* adhesion to and invasion of A549 epithelial cells. Cell viability studies using LDH and MTT assays indicated that *S. epidermidis* CFCM concentrations of 1% and 5% did not cause cytotoxic effects. Biofilm assays further confirmed that both CFCM concentrations significantly reduced biofilm formation of *S. aureus*. These findings highlight the potential of *S. epidermidis*-derived compounds for inhibiting *S. aureus* virulence and emphasize the importance of exploring skin microbiota as a source for novel antimicrobial strategies.

15. Aneuploidy induction mechanism in Ewing sarcoma **Priyani Hapugaswatta**

    Ewing sarcoma (ES) is the second most prevalent sarcoma that develops in the bones of adolescents and children. A major aberration in Ewing sarcoma is the chromosomal translocation between chromosomes t(11;22), resulting in the loss of one allele of EWSR1 and acquisition of EWSR1-FLI1 fusion gene. Trisomy 8 (~50%) is the major aneuploidy in ES tumors, and this is associated with a low survival rate in patients. To study the role of loss of one EWSR1 allele and gain of the EWSR1-FLI1 fusion gene in inducing aneuploidy, we established a conditional DLD-1 cell line that enables knockdown of proteins derived from one EWSR1 allele using Auxin-induced Degron system and expression of EWSR1-FLI1 using a Tet-on system. To investigate the effect of loss of one EWSR1 allele and gain of EWSR1-FLI1 fusion gene, cells were treated with AUX (EWSR1 knockdown derived from one allele), DOX (EWSR1-FLI1 expression), and AUX/DOX (EWSR1 knockdown and EWSR1-FLI1 expression), for 2 to 8 days. The results showed that all three-drug treated samples led to an increase in cell size. Further Fluorescence-activated Cell Sorting analysis (FACS) revealed that the majority of the small cells were diploid (2N), whereas the majority of large cells were aneuploid (>3N). Both AUX- and DOX- treated cells displayed a higher incidence of trisomy 8 compared to the control group with FISH assay. Furthermore, the AUX/DOX treated cells displayed a significantly higher incidence of trisomy 8 compared to AUX, DOX- treated, and control cells, suggesting that the expression of EWSR1-FLI1 fusion protein and EWSR1 knockdown have a cumulative effect on trisomy 8 induction. While trisomy 8 has been reported in various cancers with a poor prognosis, the induction mechanism remains unclear.

16. Knockdown of ER Quality Control Rescues Drosophila Model of the Rare Disease DPAGT1-CDG **Maya Hull**

    Glycosylation is a vital biological pathway involved in the co- and post-translational modification of proteins, affecting their function, folding, and structure. Mutations in genes involved in glycosylation lead to a rare group of disorders known as Congenital Disorders of Glycosylation (CDGs). DPAGT1-CDG is a multi-system disorder that disrupts N-glycosylation due to loss-of-function mutation(s) in the DPAGT1 gene. DPAGT1 encodes an essential enzyme responsible for synthesizing the N-glycan structure. Currently, there are no FDA-approved curative treatments for this severe condition, and approximately 80% of patients die during childhood due to significant developmental abnormalities. To identify potential drug targets, we developed a DPAGT1 disease model in Drosophila by expressing DPAGT1 RNAi in the eye, resulting in a small, rough eye phenotype. This allows us to quantitatively assess whether a treatment rescues the model by measuring eye size. Based on our previous genome-wide CRISPR screen, which identified genetic modifiers of DPAGT1, we focused on gene knockdowns in quality control pathways such as the Endoplasmic Reticulum-Associated Degradation (ERAD) pathway. ERAD is regulated by the HRD1 ubiquitin ligase complex, which comprises of HRD1, UD1/UD2, VCP, SEL1L, and others. Our findings show that knocking down these essential ERAD genes rescues the disease model. Our results also determine that disrupting certain target genes in autophagy and the Unfolded Protein Response (UPR) can rescue the disease model. Overall, these genes could serve as new therapeutic targets for DPAGT1-CDG and will be further tested with drug inhibitors in a human cell model of the disease.

17. Bioanalytical Investigation of Metabolic and Transcriptomic Profiles in a Human Tri-Culture Neuroinflammation Model **Patryk Hupert**

    Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by the deterioration and eventual death of central nervous system cells and is currently incurable. AD directly affects approximately 50 million individuals worldwide and indirectly countless families and caregivers. Growing evidence suggests neuroinflammation is a key factor in understanding neurodegenerative pathologies. Therefore, our research aims to develop tools for modeling and investigating neuroinflammation and its role in neuronal death.

    Traditional mono-culture models, models containing only one cell type, inadequately represent neuroinflammation due to their inability to capture essential cell-to-cell communication. Existing mixed culture models better reflect biological complexity but often rely on specialized techniques and costly materials that limit accessibility to researchers. To overcome these limitations, our system is made with only commercially available cells and materials, incorporating three cell types that are central to neuroinflammation, microglia (HMC3), astrocytes (HA), and neurons (SH5Y-SY).

    Imaging and cell counting analyses of the tri-culture system show that the cells are viable. A method using high performance liquid chromatography (HPLC) with diode array detection was developed to measure intracellular energy metabolites including adenine nucleotides (ATP, ADP, AMP), redox cofactors ($NAD^+$/NADH and $NADP^+$/NADPH), and related purine derivatives. RNA sequencing is used to characterize transcriptomic variations at basal conditions between the tri-culture and mono-culture, with principal component analysis to visualize sample-level variance and differential expression analyses to confirm whether variance is driven by the experimental model. Together, these methods provide valuable insights on a multi-omic level to help evaluate if such a model is viable for neuroinflammation studies.

18. Genetic adaptation alters the antibiotic susceptibility of quorum sensing (LasR)-null mutants in *Pseudomonas aeruginosa* **Sumaya Afnan Jahin**

    *Pseudomonas aeruginosa* is a multi-drug-resistant pathogen and a model for studying quorum sensing (QS), a cell-to-cell communication system involving diffusible signals (produced by LasI) and a signal-

responsive transcription factor (LasR). In *P. aeruginosa*, QS regulates virulence and antibiotic resistance and is a potential target for novel therapies. Paradoxically, chronic infection isolates often have mutations in lasR. We hypothesized that these isolates may have adaptive mutations that modulate the effects of LasR on antibiotic resistance, thereby permitting their emergence in antibiotic-treated patients. Using an experimental evolution approach, we previously identified a single-amino acid mutation in the ribosome accessory factor EF-G1A that increases the tobramycin resistance of lasR mutants but not that of an isogenic wild-type strain. Here, we expanded these studies to gentamicin. We transferred a wild-type *Pseudomonas aeruginosa* strain PA-14 population daily, and lasR mutants emerged and rapidly proliferated in the population starting at ~5 days of transfer. When populations were passaged with sublethal concentrations of gentamicin, lasR mutant emergence was delayed to ~8-20 days. Moreover, under conditions of gradually increasing gentamicin concentrations, the emergence was further delayed, typically occurring around day 20. In every case, once they emerged, they proliferated to high levels, although there was variation in the rate of proliferation. To characterize the populations, wild type and lasR mutant variants were isolated from each of the populations at the time of their collapse, and MIC assays with these variants showed no substantial difference in antibiotic resistance between most wild-type and lasR mutant isolates. However, one wild-type isolate showed a >5-fold higher MIC than its paired lasR mutant, and this pair originated from the population under increasing gentamicin selection. These results suggest that antibiotic treatment delays but does not prevent the emergence of lasR mutants. The variability of lasR emergence and MIC profiles points to the presence of secondary adaptive mutations influencing lasR selection. Future studies are aimed at identifying the underlying mutations and understanding the mechanisms driving these effects.

19. Nuclear APC maintains colon homeostasis and mitigates inflammation **Anika James**

    Adenomatous polyposis coli (APC) is critical for maintaining intestinal homeostasis. Although widely recognized for its cytoplasmic tumor suppressor functions, the roles of APC in other subcellular compartments and inflammation are less defined. To investigate nuclear APC functions, we developed a mouse model with compromised nuclear Apc import (ApcmNLS/mNLS mice). ApcmNLS/mNLS mice displayed lower levels of mucin-2 (MUC2) RNA, the primary component of the intestinal mucus barrier, and had significantly thinner colonic mucus layers. These mice were more susceptible to experimentally induced colitis than their wild-type littermates. Importantly, male ApcmNLS/mNLS mice exhibited significantly increased weight loss and higher colitis scores compared to female mice. Therefore, we hypothesize that nuclear APC promotes gut barrier integrity by upregulating MUC2 expression. In cultured human colon cells, APC positively regulates MUC2 RNA levels and inhibits NF-κB signaling. Overall, this study provides evidence that nuclear APC upregulates colonic MUC2 expression, increases the mucus barrier, and inhibits colonic inflammation. Furthermore, our results reveal notable sex-specific differences in colitis susceptibility.

20. Ultra-High-Throughput Virtual Screening & Binding Site Prediction **Andrew Johannesen**

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

21. The Computational Chemical Biology Core: A core lab **David Johnson**

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

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

With the software and expertise to perform virtual screening, protein-small molecule docking, protein/peptide modeling/docking/design, and cheminformatic analysis, the CCB is a valuable resource to enhance the productivity of biochemistry, biology, medicinal chemistry, and pharmaceutical chemistry researchers.

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

22. Initial characterization of post-embryonic gut development in the ctenophore, *Mnemiopsis leidyi*
**Nicasio Jurani**

One trait shared by most animals is the presence of a digestive system, which is used to obtain nutrients from other organisms. While the animal gut can take on many different shapes and sizes, the most common structure of the digestive system is the tripartite through-gut, which is divided into three regions: 1) the mouth (food uptake), 2) the stomach (nutrient absorption), and 3) the anus (waste removal). The through-gut enables adequate nutrient absorption and the simultaneous egestion of waste. Within the animal clade, it was thought that all non-bilaterians possessed a simple, sac-like gut, whereas the through-gut was an evolutionary innovation for bilaterians. However, it was recently shown that the non-bilaterian phylum of ctenophores possess a through-gut. With the phylogenetic position of Ctenophora at the base of the animal tree, the presence of the through-gut in these animals challenged the current evolutionary understanding of when the through-gut actually evolved. However, very little is known about the cellular and molecular aspects of how the gut develops in ctenophores. To begin to understand how the gut takes shape, we characterized the development of the endodermal canal system in *Mnemiopsis leidyi* hatchlings. Through live imaging of hatchlings at 1-day intervals, our results show that the endodermal canal system (analogous to the stomach of the tripartite system) undergoes dynamic developmental patterning over the course of 10 days. We describe major morphological timepoints of endodermal canal development, and discuss how these will provide a foundation for future experimental studies on ctenophore gut development.

23. Inhibition of HuR in Cancer Cells Enhances T Cell Activation and Sensitizes Tumors to Immunotherapy
**Sunghae Kim**

Despite the promising potential of cancer immunotherapy, most tumors remain immune “cold” and poorly responsive due to ineffective T cell activation within immunosuppressive tumor microenvironments. The RNA-binding protein HuR (ELAVL1) is ubiquitously overexpressed in various cancers and plays a key role in tumor progression, immune evasion, and therapeutic resistance. Our previous work demonstrated that blocking HuR cytoplasmic translocation downregulates PD-L1 expression, thereby disrupting immune checkpoints between cancer cells and T cells.

In this study, we showed that HuR inhibition enhances anti-tumor immunity and sensitizes cancer cells to immunotherapy. Genetic knockout of HuR in cancer cells increased T cell activation and cytokine secretion (IFN-γ, IL-6) in co-culture with human PBMCs. Treatment with KH-39, a novel small molecule HuR inhibitor, further enhanced T cell-mediated tumor cell killing. Notably, KH-39 synergized with anti-PD-1 antibody therapy in multiple syngeneic mouse models, leading to significant tumor suppression and prolonged survival.

Mechanistically, we identified CD147 as a direct target of HuR. HuR binds and stabilizes CD147 mRNA, promoting downstream p-AKT signaling that supports tumor growth and immune resistance. Both HuR knockout and KH-39 treatment reduced CD147 and p-AKT expression in breast and prostate cancer cells, inhibited proliferation and clonogenic growth, and increased susceptibility to T cell-mediated cytotoxicity. In vivo, combination treatment with KH-39 and anti-PD-1 showed greater therapeutic benefit than either agent alone.

These findings reveal that HuR inhibition not only disrupts oncogenic and immune-suppressive pathways but also enhances T cell immune responses, and improves the efficacy of cancer immunotherapies.

24. Gut Commensal Metabolites Modulate *V. cholerae* Biofilm Growth **Kate Kirby**

Microbes living in the human gut engage in complex interactions, resulting in protection of their host against pathogens. One such pathogen, *Vibrio cholerae,* infects the intestine and uses motility and biofilm formation as important virulence factors. Previously, our lab identified *Enterocloster citroniae* as a gut microbiota member that produces metabolites with a dampening effect on *V. cholerae* motility while increasing biofilm formation. This reflects an expected inverse relationship between motility and biofilm formation in bacterial behavior. Our goal is to isolate additional gut microbiota members that show biological activity against *V. cholerae*. After isolating gut microbes from fecal samples, we used ethyl acetate to extract their metabolites and performed a biofilm assay to demonstrate the effects of gut commensal extracts on *V. cholerae* biofilm formation. 41 strains were tested; of these, 10 showed a significant increase in *V. cholerae* biofilm formation while 9 showed a significant decrease. Using 16S rRNA sequencing, we have identified strains of *Flavonifractor plautii* and *Neglecta timonensis* as biofilm-inducing. Strains that did not modulate *V. cholerae* biofilm production have been identified as *Clostridium tyrobutyricum*, *Staphylococcus epidermidis*, *Cutibacterium acnes*, *Bacillus licheniformis*, and *Anaerotruncus colihominis*. Other strains have yet to be identified, including the biofilm-inhibiting strains. We are continuing to isolate, test, and identify more gut bacteria to investigate their effects on *V. cholerae* biofilm growth and motility.

25. Mechanisms of Genome Scaling in Frogs **Katherine Lenz**

Across the tree of life, genome size scales with nuclear size and cell size, yet underlying mechanisms are largely unknown. Using a combination of in vitro and in vivo approaches unique to the African clawed frog *Xenopus laevis*, we recently discovered the molecular mechanisms underlying how mitotic chromosomes scale in size during the rapid and reductive cell divisions in the early embryo. We are currently expanding our toolkit to study mechanisms of genome scaling across multiple spatial scales of 3D genome organization and in the context of early development, evolution and disease.

26. Intravenous Recombinant Human ApoE2 Protein Sex-Specifically Improves Serum Lipidomic Profile in Human ApoE4 Knock-In Mice **Yan Luo**

Apolipoprotein E (ApoE) exists in three major isoforms—ApoE2, ApoE3, and ApoE4—defined by amino acid differences at positions 112 and 158. Despite minor sequence variation, these isoforms exhibit distinct structural and functional properties with wide-ranging implications in lipid metabolism and disease risk. While ApoE4 is strongly associated with increased susceptibility to Alzheimer’s disease

and cardiovascular disease, ApoE2 is generally protective against both. However, the mechanisms underlying these opposing effects remain unclear. We investigated the metabolic impact on lipidomic compositions induced by recombinant human ApoE2 (rhApoE2) protein treatment in human ApoE4 knock-in (hApoE4KI) mice. Eight weeks of intravenous administration of rhApoE2 resulted in sex-specific alterations in serum lipid profiles. In male hApoE4KI mice, rhApoE2 significantly decreased levels of ceramides, diglycerides, and saturated/monounsaturated triglycerides while increasing polyunsaturated triglycerides. In females, rhApoE2 reduced phosphatidylethanolamines and elevated polyunsaturated triglycerides. Despite these lipid changes, lipoprotein cholesterol levels remained unchanged in both sexes following rhApoE2 treatment. Notably, compared to females, male hApoE4KI mice presented higher levels of ceramides, which correlated with lower insulin sensitivity and glucose tolerance, highlighting their potential as key mediators of ApoE4-associated metabolic dysfunction. In contrast, hApoE2KI mice, especially females, exhibited superior insulin sensitivity despite elevated LDL/VLDL levels. These findings suggest that rhApoE2 exerts beneficial, sex-dependent effects on ApoE4-mediated lipid metabolism, primarily through ceramide downregulation. Our study provides insight into the possibility that ceramides may play a more significant role in influencing metabolic health than traditional cholesterol metrics, and it supports the therapeutic potential of rhApoE2 in mitigating ApoE4-driven metabolic and neurodegenerative diseases.

27. Flow Cytometry Core: A Chemical Biology of Infectious Disease COBRE Core Laboratory **Peter McDonald**

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

28. University of Kansas Shared Resource Laboratories (SRLs) and Biomedical Research Cores **Peter McDonald**

    The University of Kansas' shared resource laboratories (SRLs), or core labs, are specialized research facilities that provide access to advanced equipment, technologies, and expert services for researchers. The core labs at KU benefit university investigators and industry partners alike. Each support lab has experienced professionals and state-of-the-art technology to support you throughout your project. Working with core research labs provides multiple benefits: (1) full-time technicians to train you to use selected equipment, (2) temporary expertise for your project when you need it, (3) expert consulting on research design and proposal development, and (4) consultation on manuscript preparation. For external partners, outsourcing research to KU's state-of-art facilities has helped our corporate partners defray costs, extend capabilities, access expert scientific advice and service, and solve problems. If you are interested in working with one of our labs, contact the director to discuss capabilities, equipment

availability, service rates and policies. The University of Kansas is committed to advancing research and academic endeavors at both KU and neighboring scientific communities. Our research core facilities offer a wide variety of services, ranging from cell sorting to electron microscopy to X-ray crystallography. Through these diverse resources, we provide access to state-of-the-art equipment and instrumentation, technical expertise, training and education all designed to support innovative, cutting-edge research.

29. The Hox gene *mab-5* controls left-right asymmetric development of the nervous system in *Caenorhabditis elegans* **Mark Yorio**

    The nervous systems of bilaterian animals including humans display left-right (L/R) asymmetries that are important in normal function and in disease states such dyslexia and schizophrenia. The genetic mechanisms specifying left-right asymmetric development remain poorly understood. In the model organism nematode worm *Caenorhabditis elegans,* the dorsal growth of the axons of the VD GABA-ergic motor neurons display left-right asymmetry, with most axons extending on the right side of the animal. Mutations in the *mab-5* gene, which encodes an Antennapedia-like Hox transcription factor, result in aberrant extension of VD axons on the left side of the animal. In previous studies, transcriptional targets of MAB-5 control L/R asymmetric migration of the Q neuroblasts downstream of MAB-5. Results in this work show that MAB-5 target genes in the Q neuroblasts also control L/R VD asymmetric axon guidance, including the Ephrin-like molecule EFN-4 and the novel, conserved kinesin molecule VAB-8. Thus, conserved transcriptional cassettes downstream of MAB-5 act in multiple neurodevelopmental processes in *C. elegans* and are likely conserved in other bilaterians including vertebrates.

30. The ABC transporter EF2223-EF2221 of *Enterococcus faecalis* imports high mannose glycans, and is dependent on a three-component signal transduction system **Abdulrahman Naeem**

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

31. Gut microbiome-derived metabolites modulate *Vibrio cholerae* interactions with host cells **Saeideh Nasiri**

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

demonstrate that small molecules produced by *E. citroniae* significantly enhance biofilm formation and reduce swimming motility in *V. cholerae*.

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

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

32. Mitochondria, Microbes and the Aging Mind **Noshin Nawar**

Mitochondria (mito) and microbes (micro) maintain conserved characteristics due to their shared evolutionary origins – with mitochondria evolving from alphaproteobacteria during the early evolution of eukaryotes. Because of these shared characteristics, therapeutics/metabolites targeting one group can impact the other (i.e., some antibiotics target shared mito-micro features). Some antibiotics can subsequently damage mitochondria, leading to mito-dysfunction. There are widespread symptoms associated with mito-dysfunction due to the multitude of functions performed by mitochondria. In addition to producing energy, mitochondria are regulators of cell death, the cell cycle, and immunity. Mito-dysfunction is therefore a core feature of many common diseases including neurodegenerative diseases like Alzheimer's disease (AD). Recent studies have also shown that alterations to the gut microbiota influence cognitive decline and actively contribute to AD progression. While both mito-dysfunction and micro-modification are associated with AD, it is unclear if they represent independent effects or if breakdown of mito-micro homeostasis leads to brain-gut dysbiosis. We hypothesize that shared mito-micro characteristics mediate brain-gut disruption and contribute to AD pathology and that some antibiotics may accelerate AD by driving mito-dysfunction. One antibiotic of interest to the AD-community is chloramphenicol which targets shared mito-micro features of protein synthesis. Prior studies show that chloramphenicol impairs mito-function in zebrafish and elevates AD genetic markers (app, tau, apoE, α-synuclein) in neuronal cultures. The effects on AD pathology in vivo remain unexplored. We are using threespine stickleback fish (*Gasterosteus aculeatus*) to test how exposure to chloramphenicol impacts AD-genetic markers in multiple mitochondrial genomic, mitochondrial physiological, and gut microbiome backgrounds. We are further testing chloramphenicol exposures at different ages and durations to disentangle chronic vs sporadic antibiotic exposures on AD-genetic markers.

33. Clusterin (CLU) as a Modulator of Neuronal Excitatory/Inhibitory Balance **Vanessa Nguyen**

Clusterin (CLU), also known as apolipoprotein J, is the third major genetic risk factor for late-onset Alzheimer's disease (AD). While CLU is well-characterized for its role in modulating amyloid-β (Aβ) aggregation, clearance, and transport—key processes in plaque formation and neuroinflammation—emerging evidence highlights broader neuroprotective functions. We investigate the impact of CLU deficiency on neuronal integrity and excitatory/inhibitory (E/I) synaptic balance in the adult mouse brain. Using 15-month-old wild-type (WT), CLU knockout ($CLU^{-/-}$) mice, employing immunohistochemistry, Western blotting, and single-nucleus RNA sequencing (snRNA-seq) to examine structural and transcriptional changes. Immunostaining for MAP2 revealed significant neuronal disorganization and

reduced neuronal density in $CLU^{-/-}$ mice. Additionally, expression levels of synaptic markers VGAT (inhibitory), VGLUT1 (excitatory) showed sex-specific alterations, indicating disrupted E/I balance. These findings were validated by Western blot analysis. snRNA-seq profiling of cortical tissue from male $CLU^{-/-}$ mice further revealed transcriptional signatures indicating hypo-excitability, including a significantly decreased ratio of the percentage of glutamatergic versus inhibitory neurons in the total cell counts. These data suggest CLU plays a critical role in maintaining neuronal homeostasis, network stability. Ongoing studies using Acridine orange assays aim to functionally quantify synaptic activity, bridging structural and molecular changes with physiological outcomes. Collectively, our findings demonstrate that CLU loss can lead to changes in neuronal structure, composition, contributing to synaptic and cognitive deficits. This study expands the understanding of CLU's role in AD pathogenesis beyond Aβ handling, pointing to its importance in synaptic integrity and identifying potential therapeutic targets for neurodegeneration intervention.

34. Up-Regulating the cGAS-STING Pathway via HuR Inhibition to Enhance Immunotherapy in Prostate Cancer **Ngoc Nguyen**

    Prostate cancer is the most common cancer type in men and the second leading cause of cancer deaths worldwide. Cancer immunotherapies have achieved significant success compared to traditional methods including chemotherapy and radiotherapy; however, they still face challenges including low response rate in patients. Therefore, discovering new approaches is crucial to enhance the treatment efficacy and patients' survival. The cGAS-STING pathway is a cytosolic dsDNA sensor that is part of the innate immune system and responds to cancer cells. Human antigen R (HuR), also known as HuA or ELAVL1, is an RNA-binding protein that has been studied as a potential target in cancer treatment. HuR is involved in various post-transcriptional regulatory processes, including mRNA splicing, maturation, nuclear export, stability, and translation. Consequently, HuR dysfunction contributes to various diseases including cancers; and ubiquitous cytoplasmic HuR levels are found in various cancer types. We hypothesize that HuR promotes cancer immune evasion by suppressing the cGAS-STING pathway, HuR inhibition can overcome the immuno-resistance and improve the response of cancer immunotherapies. HuR-mRNA inhibition using small molecule inhibitors (KHs) is expected to foster the immune system against cancer cells. This study will offer a promising strategy for cancer treatment and further research is required to understand the mechanisms on how HuR regulates the cGAS-STING pathway, hence lead the activation of immune system.

35. Assessing the HIF pathway in Ctenophores **Zach Opoka**

    Ctenophores are phylogenetically placed as the sister group to all animals. My proposed PhD project aims at studying oxygen homeostasis mechanisms in Ctenophores. The primary pathway responsible for oxygen homeostasis is the hypoxic inducible factor pathway (HIF). The HIF gene regulatory network is present in nearly all metazoans. This pathway consists of HIFa, ARNT, EGLN, and VHL. Ctenophores contain a potential HIFa gene and ARNT, but they are missing the repression/inhibitory genes: EGLN and VHL. In situ hybridization, immunofluorescence, and ChIP-seq experiments will be performed to gain insight into where HIFa is expressed, localized, and what genes it is binding to. To find pathways associated with fluctuations in oxygen, we will be performing a hypoxic exposure experiment followed by a weighted gene correlation network analysis (WGCNA). By examining oxygen related molecular responses in ctenophores, we can gain early evolutionary insight into oxygen homeostasis mechanisms.

36. Investigating the Role of the RNA-Binding Protein HuR in Pancreatic Cancer **Candice Osagie**

    Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies, marked by aggressive growth, therapeutic resistance, and poor prognosis. RNA-binding proteins (RBPs) have emerged as key regulators of gene expression in cancer by controlling mRNA stability, localization, and translation. HuR (ELAVL1), a well-characterized RBP, modulates the expression of numerous oncogenes and stress response genes. We hypothesize that HuR plays a critical role in PDAC cell

survival, proliferation, and adaptation to cellular stress. To investigate this, HuR expression was modulated using both small-molecule inhibitors and CRISPR/Cas9-mediated knockout strategies. In parallel, RNA sequencing and analysis of The Cancer Genome Atlas (TCGA) dataset were conducted to identify HuR-centered gene networks associated with tumor maintenance and progression. Ongoing efforts are focused on mapping HuR's mRNA interactome in PDAC and exploring its influence on tumor–microenvironment interactions. These findings underscore HuR's potential as a therapeutic target and support the growing recognition of RBPs as key contributors to pancreatic cancer biology.

37. Insights into How Chaperone Net Charge and Pattering Influence Nucleic Acid Folding **Gabby Perkins**

    Nucleic Acids (NAs) have roles in many major biological processes, including but not limited to translation, splicing, and DNA replication. For NAs to play their biological role, they must first form their functionally competent (i.e., native) structures. However, there are large energetic barriers NAs must overcome as they fold into these complex secondary and tertiary structures. NA binding proteins (NABPs) can help NAs adopt their native structure by transiently or specifically interacting with NA molecules. To explore the fundamental principles associated with how proteins assist folding, we systematically "re-build" NABPs from their smallest cationic constituents (e.g., ammonium→ Lys→ polyK10→ NABP). Using bioinformatics and single-molecule FRET, we have begun to uncover sequential aspects of NABPs that contribute to their chaperoning function, such as net cationic charge and charge distribution. Our results suggest that the size of cationic clusters in NABPs contributes to binding affinity and structural compaction but does not dictate folding favorability.

38. Identification of a series of pyrrolo-pyrimidine based SARS-CoV-2 Mac1 inhibitors that repress coronavirus replication **Jessica Pfannenstiel**

    Coronaviruses (CoVs) can emerge from zoonotic sources and cause severe diseases in humans and animals. All CoVs encode for a macrodomain (Mac1) that binds and removes ADP-ribose from target proteins. SARS-CoV-2 Mac1 promotes virus replication in the presence of interferon (IFN), though the mechanisms by which it promotes replication remain unknown. Mac1 inhibitors could help elucidate these mechanisms and serve as therapeutic agents against CoV-induced diseases. We previously identified compound 4a, a pyrrolo-pyrimidine that inhibited Mac1 activity in vitro at low micromolar levels. Here, we determined the binding mode of 4a by crystallography, further defining its interaction with Mac1. However, 4a did not reduce CoV replication, which we hypothesized was due poor cellular permeability. To test this hypothesis, we developed several hydrophobic derivatives of 4a and identified four compounds that both inhibited Mac1 in vitro and inhibited murine hepatitis virus (MHV) replication: 5a, 5c, 6d, and 6e. Furthermore, 5c and 6e inhibited SARS-CoV-2 replication only in the presence of IFN$\gamma$, similar to a Mac1 deletion virus. To confirm their specificity, we passaged MHV in the presence of 5a and 5c to identify drug-resistant mutations and we identified an A1438T/G1439V double mutation in Mac1 following passaging with 5a, and a G1386E mutation following passaging with 5c. We engineered recombinant viruses with these mutations and found they had enhanced replication compared to WT virus when treated with these compounds, demonstrating the specificity of these compounds during infection. Furthermore, the A1438T/G1439V recombinant virus was highly attenuated in vivo, indicating that drug-resistance emerged at the expense of viral fitness.

39. RNA binding protein Musashi1 regulates mTOR pathway, potentially controlling growth of the intestinal epithelium **Bikash Pokhrel**

    Colorectal cancer (CRC) is the second leading cause of cancer-related death, affecting 1 in every 24 people. CRC develops due to an imbalance in cell division, differentiation, and death in the epithelial cells lining the intestine. Musashi1, an RNA-binding protein, is exclusively expressed in stem and dividing cells and is essential for their maintenance and fate to subsequent differentiation. Our published research showed that ubiquitous overexpression of Musashi1 in mice resulted in reduced growth of the organism and various organs. We employed bulk RNA sequencing and in-vitro studies to

determine the mechanism underlying this growth defect. Our studies revealed that Musashi1 inhibits growth by regulating the mTORC1 pathway. The mTORC1 pathway is central to cellular growth, as it controls both anabolic processes like protein and lipid synthesis and catabolic processes like autophagy. Immunofluorescence studies revealed that Musashi1 protein and mTORC1 activity are mutually exclusive in cells of the intestine, consistent with a key role for this interaction in determining the fate of stem and dividing cells. Our current work aims to identify the RNA targets of Musashi1 that regulate the mTORC1 pathway. Given that mTORC1 and Musashi1 are often dysregulated in CRC, our study may lead to improved treatment strategies.

40. PTPN22 Impacts the B Cell Landscape During Chronic Virus Infection **Macie Proctor-Rose**

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

41. Riboregulatory sequence 5BSL3.2 modulates the conformations of 3′X RNA in hepatitis C virus **Ethan Rogers**

    The hepatitis C virus (HCV) genome is a positive-sense, single-stranded RNA that contains many structured regions critical to viral propagation. This work focuses on a cis-regulatory element, 5BSL3.2, which has been shown to interact with the 3′X RNA of HCV. Recent work has detailed the structural dynamics of 3′X, illustrating its slow interconversion within a bi-conformational equilibrium. In this presentation, we detail the effects of 5BSL3.2 on the 3′X conformational equilibrium via single-molecule FRET. By applying a cyclic four-state model, we determine the coupled equilibrium constants of conformational interconversion and the binding affinities of the respective 3′X conformations. Our results demonstrate that 5BSL3.2 can bind to both 3′X conformations and biases the conformational equilibrium towards one of the intrinsic structures of 3′X. This work supports an emerging hypothesis that suggests the 3′X-5BSL3.2 interaction is a part of a riboregulatory network that controls viral replication and translation.

42. Infectious Disease Assay Development Core: High Throughput Screening Laboratory at the University of Kansas **Anuradha Roy**

    The overall goal of the IDAD Core is to provide expertise, facilities, services, and training in the area of HTS assay design, development, validation, small and large-scale screening for whole cell based or biochemical infectious disease targets. The IDAD core is an extension of the University of Kansas High Throughput Screening Laboratory which is a fee-for-service, state-of-the-art facility dedicated to providing academia, not-for-profit institutions, biotech, and pharmaceutical industries with exceptional assay development, high throughput screening and data mining services at economical rates. The staff has experience in executing cell-based, biochemical, siRNA as well as high content screening

campaigns against a plethora of target classes. The laboratories are equipped with cutting-edge liquid handling and signal detection instrumentation for increasing throughput and precision of screening campaigns. Clients have the option of using our collection of 395,000 compounds and/or a client's own chemical library. KU-IDAD/HTS lab further leverages the strengths of the medicinal chemistry/ computational modeling cores under CoBRE Chemical Biology of Infectious diseases (CBID) program to support your tool/lead discovery research.

43. Fly Viral Atlas: Cellular tropism of Drosophila RNA viruses and their impact on the host **Nilanjan Roy**

    Drosophila RNA viruses are diverse and often persist in wild and lab populations, yet their cell tropism is poorly understood. In the FlyCellAtlas (represents comprehensive Drosophila single-cell transcriptome) single-cell RNA-seq data, we detected four natural RNA viruses: Nora virus, Drosophila A virus (DAV), Drosophila C virus (DCV), and Newfield virus. Nora and DAV were abundant and widespread across tissues, while DCV and Newfield virus showed high, tissue-specific expression in oenocytes and fat body, respectively. Transposable elements (TEs) are traditionally associated with the germline, but recent studies—and our data—show they are also expressed in somatic cells. Using FlyCellAtlas, we found that distinct somatic cell types express specific TE subtypes, indicating regulated and cell-type-specific TE activity often overlooked in transcriptomic studies. Moreover, we found that TE expression is significantly upregulated in RNA virus infections. Here, we present Fly Viral Atlas (flyviralatlas.shinyapps.io/home), a single-cell atlas of RNA virus and transposable element (TE) expression in Drosophila, providing new insights into viral tropism and TE dynamics across tissues.

44. PARP14 in Host-Virus Interaction: Identifying Domain(s) Required for Viral Replication **Elle Saenjamsai**

    PARP14 is an ADP-ribosyltransferase containing a multi-domain structure that is involved in cellular processes such as DNA repair, inflammation, and immune regulation. Notably, PARP14 is the largest PARP with five different types of domains. PARP14 regulates viral replication, suggesting a role in host-pathogen interactions. However, its domain-specific contributions to antiviral activity remain unclear. We have found that PARP14 promotes IFN-I responses and restricts the replication of mutant Murine hepatitis virus (MHV), mutant SARS-CoV2 and herpes simplex virus 1 (HSV-1) in different manners. PARP14, on the other hand, promotes the replication of Vesicular Stomatitis virus (VSV), demonstrating that it plays both pro- and anti-viral roles. We hypothesize that these contradictory effects are caused by different domain(s) of PARP14. To test this, we will identify the domains required for IFN-I production following poly(I:C) stimulation, as well as the domain(s) that restrict HSV-1 replication. We will use a series of PARP14 truncation mutants in A549 PARP14 knockout cells. IFN-I response will be evaluated using qPCR and ELISA, while viral replication will be quantified by plaque assays. Our study aims to clarify the mechanisms by which PARP14 regulates host-virus interactions, offering insights into potential therapeutic targets for viral infections.

45. Elucidating quorum sensing-dependent crosstalk in a Burkholderia-Pseudomonas coinfection model **Ahnaf Tahmid Saqif**

    Quorum sensing enables bacteria to sense and respond to changes in population density, resulting in coordinated group behavior through regulation of gene expression. One type of system common to many proteobacteria involves the production and response to a small signal molecules, which are acyl-homoserine lactones (AHLs). The signals facilitate changes in gene expression by interacting with and binding to a cytoplasmic receptor, which is a transcription factor. The signals can vary in their acyl group side chain, which is thought to confer fidelity of communication with kin. However, some bacteria can respond to non-native signals, suggesting the potential for interspecies crosstalk. In a recent study, it was demonstrated AHL-dependent crosstalk between two bacteria- *Pseudmonas aeruginosa* and *Burkholderia multivorans* – that increased mortality in a C. elegans infection model compared to mono infections. The current study focuses on studying the LuxI/LuxR type quorum sensing system of *B.*

*multivorans* to address the promiscuity of this system. First, we established growth kinetics of *B. multivorans* and confirmed its ability to reach sufficient cell density for quorum sensing activation. Using cell-based biosensors, we quantified its AHL, N-octanoyl homoserine lactone (C8-HSL), and assessed C8-AHL-dependent regulation of a downstream gene target cepI. Ongoing experiments aim to determine the responsiveness of *B. multivorans* to C8-HSL and the AHLs produced by *P. aeruginosa*, C4-HSL and 3-oxo-C12-HSL. These studies will clarify whether *B. multivorans* exhibits promiscuous signal sensing as suggested by the co-culture models. Together, our work contributes to a deeper understanding of *B. multivorans* quorum sensing dynamics in monoculture and sets the stage for dissecting how interspecies AHL recognition might influence virulence and competitive fitness in polymicrobial environments.

46. Interactions between the *Pseudomonas aeruginosa* Las quorum-sensing system and stringent response reveal distinct mechanisms of resistance to ceftazidime and piperacillin **Vanessa Schmidt**

    In *Pseudomonas aeruginosa*, the acyl-homoserine lactone (AHL)-dependent las quorum-sensing system enhances resistance to multiple antibiotics, including the clinically important β-lactam ceftazidime. β-lactams susceptibility is also influenced by the stress-inducible stringent response system, which increases detoxification of reactive oxygen species (ROS) in non-growing cells to maintain membrane integrity and mitigate antibiotic internalization. Here, we examine the interaction of LasR with stringent response (SR) in shaping resistance to the β-lactam antibiotics ceftazidime and piperacillin. Our results showed that disrupting LasR decreases resistance to ceftazidime but not piperacillin. LasR also increases ROS levels and uptake of ethidium bromide, a proxy for measuring membrane permeability, similar to that of the SR system. We could restore ceftazidime resistance and membrane resistance in the ΔlasR mutant by chemically or enzymatically detoxifying ROS. These results suggest LasR increases ceftazidime resistance through a shared pathway of ROS detoxification and protecting membrane integrity. In support of this idea, concurrently disrupting both the LasR and SR systems resulted in a non-additive effect on ceftazidime resistance that was similar to that of disrupting the individual systems. In contrast, we observed a different pattern for piperacillin. Individually disrupting LasR or SR had little to no effect on resistance, while disrupting both systems caused >10-fold decrease in resistance, revealing synergistic effects of these two systems that did not correspond to membrane permeability changes. These results indicate LasR and SR confer piperacillin through synergistic but different mechanisms potentially unrelated to membrane permeability. Together, our work reveals new mechanistic insights into how LasR and stringent response contribute to β-lactam antibiotic resistance that may be relevant to understanding the variables that influence *P. aeruginosa* evolution in complex environments, including infections.

47. Molecular Mechanisms of Resistance to Topoisomerase-Targeting Drugs in Adenomatous Polyposis Coli-Mutant Colorectal Cancer **Azeem Talabi**

    Cancer accounts for 1 in 5 deaths worldwide, with colorectal cancer (CRC) ranking as the third most common and the second leading cause of cancer-related deaths. While surgery is effective for early-stage CRC, metastatic cases depend on chemotherapy, which is often hindered by drug resistance. Approximately 80% of CRC cases are driven by mutations in the tumor suppressor gene Adenomatous Polyposis Coli (APC), a key regulator of various cellular processes, notably the Wnt/β-catenin pathway. Topoisomerase II (Topo II), an enzyme essential for DNA metabolism, is frequently overexpressed in CRC due to uncontrolled proliferation, making it an attractive therapeutic target. However, APC-mutant CRCs show increased resistance to Topo II-targeting drugs compared to other drug classes. Our previous work identified a functional interaction between APC and Topo II, linking it to cell cycle checkpoint control. Yet, the precise mechanism by which APC modulates Topo II drug sensitivity remains unclear. In this study, we use human colorectal cell lines with altered APC expression and integrate techniques such as flow cytometry, viability assays, immunoblotting, and DNA-protein crosslink assays to examine the differential DNA damage response triggered by Topo II-targeting drugs based on APC expression. Furthermore, we explore whether this regulation occurs through Wnt-dependent or Wnt-independent pathways. This research aims to understand how APC mutation or loss

confers resistance to Topo II-targeting agents and explores combination therapies to restore drug effectiveness in APC-deficient colorectal cancer.

48. University of Kansas – Preclinical Core Facilities **Xiaoqing Wu**

    The Experimental and Preclinical Imaging Core provides investigators with the ability to optimize drug treatments in small animals and cell lines, including both small molecule and nanoparticle drug delivery. The capabilities of the near-infrared and bioluminescence imaging services are compatible with most small animal models. These services allow investigators the opportunity for in vivo target validation and dynamic analysis of mechanism of action of novel molecular therapeutics which will advance drug discovery projects more efficiently from bench to bedside. In addition, investigators will learn more about the molecular mechanisms associated with different diseases.

    The Biological Irradiation research laboratory provides investigators the ability to examine the effects of radiation treatment upon particular models such as yeast, fruit flies, nematodes, zebra fish, mammalian cells, and small animals. This provides investigators the capability to understand the effect of ionizing radiation on critical cellular responses, such as DNA repair, cellular proliferation and inflammation. In addition, investigators will be able to develop novel therapies using combination treatments of irradiation and small molecules.

49. Defining the impact of ApoE isoforms on APP metabolism in humanized Abeta mouse models **Pranali Yadav**

    Alzheimer's disease (AD) is the most common form of dementia. It exists in two primary forms: familial and sporadic. Sporadic AD, which represents approximately 95% of the total AD population, continues to remain understudied. Apolipoprotein E (ApoE) is present in three major isoforms: ApoE2, ApoE3, and ApoE4. ApoE2 is found to be neuroprotective, and ApoE4 is recognized as the strongest genetic risk factor for sporadic AD. Amyloid precursor protein (APP) is a type I transmembrane protein and is metabolized by two major pathways: the amyloidogenic and non-amyloidogenic pathways. Recent studies have identified a third metabolic pathway, known as the η-secretase pathway. Although sporadic AD accounts for the majority of cases, most studies have focused on transgenic mouse models carrying familial AD mutations. To address this gap, we generated double knock-in mouse models by crossing humanized ApoE isoform knock-in mice with humanized wildtype Aβ (hAβwt) knock-in mice, resulting in hApoE2/hAβwt, hApoE3/hAβwt, and hApoE4/hAβwt expressing mice. We investigate age and ApoE isoform dependent differences in APP metabolites across these mouse strains using Western blot analysis. Preliminary findings suggest increased η-CTF expression with aging in ApoE2 and ApoE3 genotypes, while the η-CTF metabolic pathway appears to be comparably active at 5 months compared to 15 months in the ApoE4 genotype. Moreover, we will be examining the expression and activity of key enzymes involved in Aβ production and degradation. This study aims to elucidate ApoE isoform-specific pathways of APP metabolism and metabolites associated with sporadic AD, providing insights into novel therapeutic strategies.

50. AHL-specific global gene regulation by a promiscuous receptor in *Chromobacterium subtsugae* **Eryk Yarkosky**

    Acyl-homoserine lactone (AHL) quorum sensing enables bacteria to sense and respond to changes in population density. These systems involve an AHL synthase and a receptor that functions as a transcription factor. Upon AHL binding, the receptor undergoes a conformational change that enables interaction with a conserved recognition sequence in the promoter of its gene targets, activating dozens of genes. AHL-receptor interactions are generally thought to be selective, ensuring kin-specific communication. However, some receptors respond to a broader range of AHLs, raising the possibility of interspecies crosstalk. Previous studies of promiscuous receptors suggest native AHLs elicit similar but more robust gene activation than non-native AHLs, but most have focused on one or a few gene

targets. How non-native AHLs impact broader gene expression remains an open question. Here, we investigated this question using the soil bacterium *Chromobacterium subtsugae*, which encodes a single AHL receptor, CviR. CviR responds to both its native signal, C6-HSL, and non-native signals such as C8-HSL. Using transcriptomics, we compared gene expression in response to these two AHLs and found that each activates overlapping but distinct regulons. We generated dose-response experiments with selected gene targets and found gene-specific differences in sensitivity to each AHL, with some promoters more strongly activated by C6-HSL and others by C8-HSL. Similar gene-specific responses were observed in a heterologous host expressing CviR, suggesting differential activation is due to CviR. Our findings support a model in which different AHLs elicit distinct CviR conformations that bias promoter sequence preference and/or transcriptional output. This study highlights how receptor promiscuity may allow bacteria to alter gene expression when non-native AHLs are detected, potentially enabling flexible behavioral responses in polymicrobial communities.

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