The University of Kansas

Department of Molecular Biosciences

2026

Molecular Biosciences Departmental 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

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Molecular Biosciences Graduate Student Organization

The University of Kansas Cancer Center

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Center for Molecular Analysis of Disease Pathways

2026 Molecular Biosciences Departmental Symposium

Friday, August 21st, 2026

Location: Burge Union Forum CD

Opening Activities

8:30 AM	Poster set-up & refreshments
9:00 AM	Welcome

Faculty Talks

9:10 AM	Dr. Allie Graham Tales of Loss: understanding the role of Pseudogenization in adaptation to environmental pressures
9:40 AM	Dr. Hans Dalton Robust or bust: studying rare diseases and gene duplications in fundamental pathways
10:10 AM	Dr. Rosana Ferriera Microbial competition on the skin: limiting pathogen virulence
10:40 AM	Break

Keynote Speaker

11:00 AM	Dr. Brian Lazzaro, Department of Ecology and Evolutionary Biology, Cornell University Antimicrobial peptide function and control
12:00 PM	Lunch

Student Talks

1:00 PM	CJ Gormly A novel non-apoptotic role of core programmed cell death pathway components to direct Q neuroblast migration
1:20 PM	Tolulope Iorwuese Ade Defining the mpt phosphotransferase system of *Enterococcus faecalis*
1:40 PM	Macie Proctor-Roser *Ptpn22 r*egulates B cell antiviral response by modulating IFNy signaling
2:00 PM	Pradtahna (Elle) Saenjamsai Mac1 deletion creates a live-attenuated SARS-CoV-2 vaccine with broad protection
2:20 PM	Break

Poster Sessions

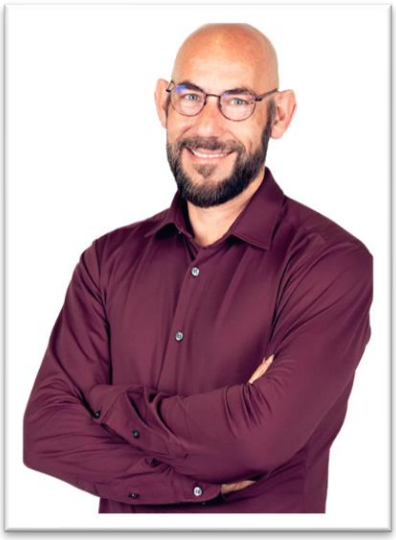
2:30 – 3:15 PM	Poster Session I
3:15 – 4:00 PM	Poster Session II

Picnic & Social

5:30 PM	Picnic & Social at Holcom Park

Keynote Speaker

Reciprocal Dynamics Governing Outcomes of Bacterial Infection in *Drosophila melanogaster*



Dr. Brian Lazzaro

Liberty Hyde Bailey Professor

Department of Ecology and Evolutionary Biology, Cornell University

Antimicrobial peptides (AMPs) are an essential component of the innate immune responses of plants and animals. AMPs are constitutively produced in barrier tissues and their systemic production can be rapidly and massively induced by bacterial infection. However, this production can be costly, and some bacteria have mechanisms for defending themselves against host AMPs. Working with a model insect host, *Drosophila melanogaster*, we are studying the dynamic interplay between the host immune response to infection and bacterial defenses against host immunity, as well as how these dynamics are altered by competing physiological demands on the host. In particular, we find that endocrine-regulated investment in reproduction places physiological strain on *D. melanogaster* females, which in turn compromises their immune defense. We also find that some AMPs are unexpectedly specific in their antibacterial activity, and that both living and dead bacterial cells have mechanisms for counteracting AMPs. The joint evaluation of both host and pathogen demonstrates a complex dynamic between the two, where small variations on either side can mean the difference between host survival or death.

Faculty Talks

Tales of Loss: Understanding the Role of Pseudogenization in Adaptation to Environmental Pressures

Dr. Allie Graham

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

The invasion of specialized ecological niches can cause drastic changes to selection regimes, resulting in genomic and phenotypic transformation. High-altitude habitats offer an excellent opportunity to investigate the genetic basis of local adaptation, as the repeated specialization of multiple lineages for high altitude has produced striking examples of convergent evolution, adaptation, and changes in their underlying genes. Although enlightening, this focus on adaptation has left aspects of evolution in high-altitude locations understudied - including the role of gene loss and pseudogenization, maladaptation and trait loss, and physiological aspects outside of respiration and gas exchange. To characterize how mammals responded to high altitude in a new, unbiased way, we screened the genomes of species living exclusively at high altitude (>1,000–1,500 m) and their lowland relatives for inactivated pseudogenes or lost genes. Genes that convergently lost function in high-altitude species were highly enriched for olfactory receptor (OR) genes. In addition to OR loss, cranial endocasts show the brains of high-altitude species have on average smaller olfactory bulbs relative to lowland relatives. Together, these repeated evolutionary outcomes suggest a general relaxation of constraint on olfaction at altitude, perhaps due to reduced odorant diversity in high-altitude environments or reduced effectiveness of mammalian olfactory physiology in thin, dry, or cold air.

Robust or Bust: Studying Rare Diseases and Gene Duplications in Fundamental Pathways

Dr. Hans Dalton

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

The Dalton Lab studies genetic pathways associated with rare diseases and gene duplications. Some rare diseases stem from "fragile" pathways that lack compensatory mechanisms when failure (e.g. mutation) occurs. We create models of these diseases to find new interventions for rescuing them. Furthermore, the underlying reason for a rare disease pathway often funnels into a small number of shared mechanisms. We try to exploit these shared mechanistic "linchpins" to generalize treatments to more than one rare disease. Finally, we characterize gene duplications of human disease genes to understand their function. If duplications of disease pathway genes exist, could these compensate for the loss of those genes, thereby increasing robustness of the pathway? Here I will describe these three research pillars of the lab and talk about progress on each of them within glycosylation and mitochondrial disease pathways.

Microbial Competition on the Skin: Limiting Pathogen Virulence

Dr. Rosana Ferriera

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

The skin is colonized by hundreds of bacterial species that interact with each other and with transient species that eventually reach this body site. We seek to better understand these interactions, focusing on how they affect microbial community establishment and protect us against pathogens. We showed that *Cutibacterium acnes*, a key member of the human skin microbiome, secretes molecules that inhibit *Staphylococcus lugdunensis* biofilm formation without affecting planktonic growth. *C. acnes*-derived molecules also significantly reduced *S. lugdunensis* adherence to and invasion of human epithelial cells, as well as adhesion to keratinocytes. Transcriptomic analysis revealed repression of genes involved in *S. lugdunensis* purine biosynthesis and induction of the autolysis negative regulators, *lrgA* and *lrgB*. Functional assays confirmed that exposure to *C. acnes* molecules inhibits autolysis and extracellular DNA (eDNA) release and decreases intracellular guanine levels in *S. lugdunensis*. Our data indicate that *C. acnes* molecules inhibit *S. lugdunensis* biofilm formation by depleting the intracellular guanine pool, leading to repression of autolysis, and reduced eDNA release, a key component of biofilm structural integrity. These findings underscore the importance of interspecies microbiome interactions in pathogen exclusion.

Student Talks

A novel non-apoptotic role of core programmed cell death pathway components to direct Q neuroblast migration

CJ Gormly

Neuroblast migration is a critical step in the formation of a functional nervous system. C. elegans are an excellent model to study this migration. The neuroblast QL is born in the posterior region of the worm between the V4 and V5 hypodermal seam cells. QL first migrates a short distance posteriorly and divides atop the V5 seam cell. The Wnt ligand EGL-20/Wnt is expressed by muscle cells near the anus. EGL-20 first prevents default anterior migration and then drives expression of the Hox transcription factor MAB-5 through canonical Wnt signaling. MAB-5 maintains the pause in migration and then drives posterior migration in the QL descendants. MAB-5 is both necessary and sufficient for posterior migration. The mechanism that EGL-20 and MAB-5 use to prevent anterior migration so posterior migration can take place is not well understood. Downstream targets of MAB-5 were identified by RNA-seq on Q-cells from mab-5 gain of function and wild-type animals. One gene that was upregulated in the mab-5 gain of function was the initiator of the programmed cell death pathway, egl-1. Loss of function alleles of egl-1 and the core components of the canonical programmed cell death pathway, ced-4/APAF1 and ced-3/Caspase-3, were screened for PQR migration defects. ced-4 and ced-3 mutants had significant failures in posterior PQR migration. egl-1 and ced-4 mutants also enhanced the aberrant anterior PQR migration in egl-20(mu39)/Wnt and bar-1(mu63)/b-catenin mutants. Mutations in egl-1, ced-3 and ced-4 also strongly suppressed mab-5 gain of function phenotype. FLI-1, a member of the gelsolin family of actin cytoskeleton regulatory proteins, was identified as a possible downstream target of CED-3. fli-1 mutants exhibited significant failures in QL posterior migration, enhanced the aberrant anterior migration in the egl-20 hypomorphs, and suppressed the mab-5 gain of function. Pathway analysis revealed that fli-1; ced-3 double mutants resembled ced-3 alone, indicating that they act in the same pathway. Together, these results suggest that the canonical programmed cell death pathway acts downstream of Wnt signaling during both the anterior/posterior migration decision in QL.a and drive posterior migration. This work uncovers a novel non-apoptotic role of the canonical programmed cell death pathway to direct migration, likely through caspase dependent regulation of actin cytoskeleton regulatory proteins to prevent default anterior QL.a migration.

Defining the Mpt phosphotransferase system of *Enterococcus faecalis*

Tolulope I. Ade and Lynn E. Hancock

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

Enterococcus faecalis is a metabolically versatile bacterium that has been reported to encode at least 46 different phosphotransferase systems (PTS), 6 of which are regulated by the alternative sigma factor 54, σ54 (RpoN) that recognizes the -24/-12 promoter sequence. These RpoN-regulated PTSs (Cel1, Cel2, Dga, Gfr, Mpt, and Xpo PTSs) are also regulated by bacterial enhancer binding proteins (bEBPs) that belong to the LevR family of transcriptional regulators - CelR, DgaR, GfrR, MptR, and XpoR. Of the 6 RpoN-regulated PTSs, the Mpt PTS is the most studied in E. faecalis and has been shown to contribute to infection outcome in several animal disease models. We have shown that the Mpt PTS plays a role in the uptake of rapidly metabolised sugars including glucose, mannose, glucosamine, and N-acetylglucosamine and the uptake of these sugars is dependent on both RpoN and MptR. We also show that these sugars induce the mptB promoter (the first gene in the mpt operon) in a RpoN- and MptR-dependent manner. Bioinformatic analysis predicts that the mpt operon contains four open reading frames – mptB (EIIB), mptA (EIIAB), mptC (EIIC), and mptD (EIID). More detailed analysis of this operon identified a second EIIB domain fused to the EIIA domain of MptA and shares ~ 50% amino acid sequence similarity with MptB. Mutational and complementation studies of these genes shows that the EIIB domain of MptA is important for growth in glucose, mannose, and glucosamine while MptB plays a role in the uptake of galactose.

Ptpn22 Regulates B Cell Antiviral Response by Modulating IFNg Signaling

Proctor-Roser, Macie A.1*, Cockerham, Tammy R.1, and Orozco, Robin C.1

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*Presenting Author

B cell Receptor (BCR) and Interferon-g Receptor (IFNgR) signaling govern B cell functions essential for antiviral immunity. Defining how these pathways are regulated may reveal mechanisms to enhance protective humoral responses. Ptpn22, associated with autoimmunity, encodes the immune cell-exclusive tyrosine phosphatase PEP. Although PEP has been implicated in BCR signaling, published findings are contradictory, and its role in IFNgR signaling remains largely unexplored. Previous studies showed PEP-deficient (PEP-null) mice infected with chronic virus LCMV-cl13 clear infection, whereas wildtype (PEP-WT) do not. Enhanced viral control is accompanied by significantly higher anti-LCMV-cl13 antibody titers, suggesting PEP regulates antiviral B cell responses. We hypothesize that PEP primarily regulates IFNgR rather than BCR signaling, leading to altered T:B cell interactions, increased antibody production, and viral clearance in PEP-null mice. Using phospho-flow cytometry, we investigated BCR and IFNgR signaling kinetics in PEP-WT and PEP-null B cells following receptor stimulation. Results showed no difference in pSYK phosphorylation or overall BCR signaling. In contrast, PEP-null B cells exhibited increased pSTAT1 phosphorylation, consistent with enhanced IFNgR signaling. To determine biological consequences, we immunophenotyped B cell subsets in spleen, lymph nodes, and bone marrow of naïve mice and following LCMV-cl13 infection. While PEP did not affect B cell development or major differentiation subsets, PEP-null mice exhibited germinal center-derived antibody-secreting population expansion. Finally, in coculture, we found that PEP-null B cells more effectively activated CD4 T cells and promoted TFH differentiation. Collectively, these findings define a role for Ptpn22 and PEP in regulating humoral immunity during chronic viral infection.

SARS-CoV-2 ΔMac1 infection induces a robust immune response with complete protection against both homologous and heterologous SARS-CoV-2 variants

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1 Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, OK, USA.

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The ongoing challenge in SARS-CoV-2 vaccine development is achieving durable protection against new emerging variants, as viral evolution can reduce vaccine effectiveness. While current vaccines effectively prevent severe disease, they provide limited control of transmission and waning protection against emerging variants. This highlights the need for vaccine strategies that induce broad, long-lasting immune protection. Live-attenuated vaccines (LAVs) induce strong immune responses against multiple viral antigens, and many are known to prevent transmission and provide long-lasting immunity. Here, we evaluated a SARS-CoV-2 LAV candidate with a complete macrodomain 1 deletion (ΔMac1), a virulence factor involved in immune evasion. Using K18-hACE2 transgenic mice, we found that ΔMac1 induces robust interferon response while causing little to no disease following a lethal dose infection, indicating its potential as a LAV candidate. Mice were immunized intranasally with ΔMac1, boosted, and challenged with lethal doses of wild-type or emerging SARS-CoV-2 variants. Vaccine safety, immunogenicity, and protective efficacy were evaluated. ΔMac1 infection was non-pathogenic, with no clinical disease and minimal lung pathology, demonstrating a strong safety profile. ΔMac1 vaccination induced robust, polyfunctional virus-specific CD4+ and CD8+ T cell responses targeting structural and accessory proteins in the lung and systemic compartments, alongside high-titer SARS-CoV-2-specific antibodies capable of cross-neutralizing multiple variants. These responses resulted in complete protection from lethal challenge and enhanced viral clearance from the lungs, indicating high vaccine efficacy. Together, these findings demonstrate that Mac1 deletion can safely elicit broad and durable immunity, supporting LAVs as a promising approach for protection against current and future SARS-CoV-2 variants.

Poster Session Roster

Odd numbers: Poster session 1 (2:30pm-3:15pm)

Even numbers: Poster session 2 (3:15pm-4pm)

No.	Presenter	Title
1	Tatum P. Aikin	Autoimmunity-associated allele of Ptpn22 distinctly modulates different toll-like receptor responses in bone marrow derived macrophages.
2	Lane Anaya	Hypoxia tolerance in natural high-altitude populations of *Drosophila melanogaster*
3	Alec M. Bevis	The Autoimmunity-Associated Minor Allele of PTPN22 Accelerates NK Cell Development and Augments Effector Function.
4	Alfred Buabeng	The RNA-binding protein HuR regulates CD147 glycosylation in Triple Negative Breast Cancer (TNBC).
5	Justin Carbone	Computational Investigation of Import Mechanisms of Bacterial Toxin Colicin E1 Through the Outer Membrane of *E. coli*.
6	Asbin Chand	Haspin Regulates the Checkpoint Monitoring TopoII Decatenation Activity to Maintain Chromosome Stability.
7	Mollie Coffey	PTPN22 Alternative Allele Changes the Mechanisms of T Cell Mediated Weight Loss.
8	Andres Cordova	Elucidating Colicin E1 Mechanism of Action.
9	Larissa da Silva Ferreira	Anti-virulence Activity of Host Inflammatory Cytokines IL-1β and IFN-γ Against *Salmonella enterica.*
10	Ayushee Dasgupta	Role of C-Terminal Domain Phosphorylation in Topoisomerase IIα During Mitosis.
11	Andrew Daufel	Catanionic Detergent Vesicles as a Model Linking Protomembranes to Modern Membranes.
12	Tessa K. Eads	Mapping autosomal supression to meiotic drive.
13	Piero Rodolfo Espinel Cabrera	Capturing the host ADP-ribosylome to identify host factors that restrict coronavirus replication.
14	Cael Gorman	Diversification of heterochromatin genes across polyploid *Xenopus* species.
15	Haider Warraich	University of Kansas Nanofabrication Facility: Equipment and Capabilities.
16	Jennifer Hackett	Genome Sequencing Core – KU-Lawrence.

17	Kiana Hajiarbabi	Pyroglutamic Acid Contributes to the Anti-Virulence Activity of *Staphylococcus epidermidis* Against *Staphylococcus aureus.*
18	Danny Jack	Three-Dimensional Quantification of Heterochromatin Domain Organization During Early *Xenopus* Development.
19	Sumaya A. Jahin	Antibiotic selection shapes quorum sensing evolution through adaptive mutations in *Pseudomonas aeruginosa.*
20	Andrew M. Johannesen	Ultra-High-Throughput Virtual Screening and Binding Site Prediction.
21	David K Johnson	The Computational Chemical Biology and Molecular Modeling Core.
22	Peter R. McDonald	Flow Cytometry Core: A Chemical Biology of Infectious Disease COBRE Core Laboratory.
23	Alexis M. Michalski	Investigating the Genetic Impact of Flock House Virus in *Drosophila melanogaster.*
24	Ella Miller	Host-derived Interferon-gamma modulates *Salmonella enterica* virulence and host cell interactions.
25	Ishita Mirchandani	Contribution of PEP-null and PEP-R619W Antigen-Presenting Cells in enhancing CD4 T cell function during chronic viral infections.
26	Tymber Moody	Identification and analysis of repurposed therapeutics for the rare mitochondrial disease, WARS2 deficiency, using a *Drosophila melanogaster* model.
27	Abdulrahman M. Naeem	The ABC transporter EF2223-EF2221 of *Enterococcus faecalis* imports high mannose glycans, and is dependent on a three-component signal transduction system.
28	Saeideh Nasiri	Gut microbiome-derived metabolites regulate *Vibrio cholerae* biofilm formation and host inflammatory responses.
29	Ngoc Huan Nguyen	Up-Regulating the cGAS-STING Pathway via HuR Inhibition in Cancer Cells.
30	David Noblett	Toward Metal Binding Site Design Using a Protein Language Model.
31	Lillian O'Donnell	Evolution of resistance to *Candida auris* yeast among *Drosophila* species.
32	Aidan O'Hara	Metabolites produced by bacterial skin microbiome members disrupt *Staphylococcus aureus* biofilm formation.
33	Candice Osagie	RNA-sequencing data of differential gene expression following HuR inhibition across multiple cancer cell lines.
34	Sarayu Pentapati	Functions of 3D Genome Organization.
35	Bikash Pokhrel	Musashi1 regulates growth of the mouse intestinal epithelium.
36	Ethan P. Rogers	Intragenomic RNA-RNA interactions modulate 3′X RNA conformations in hepatitis C virus

37	Anuradha Roy	The University of Kansas High Throughput Screening/Infectious Disease Assay Development Laboratory.
38	Bhawana Samal Magar	Neuronal Regulation of Pulmonary Endothelial Function During Carbapenem-Resistant *Klebsiella pneumoniae* Infection.
39	Ahnaf Tahmid Saqif	Strain background is a key determinant of quorum sensing-dependent interspecies crosstalk in *Pseudomonas aeruginosa.*
40	Vanessa M. Schmidt	A genetic screen to identify mutations that modulate the effects of quorum sensing on antibiotic resistance in the bacterial pathogen Pseudomonas aeruginosa.
41	Anam Shaikh	PEP-R619W Differentially Modulates IFN-I Signaling to Enhance Antiviral Immunity.
42	Azeem Talabi	APC loss enhances proteasome dependent repair of Topoisomerase II poison–induced DNA damage.
43	Chukwuma G. Udensi	The Microbiota-Derived Metabolite 3,4-Dimethylbenzoic Acid Inhibits *Salmonella enterica* Virulence Independently of Two-Component Systems.
44	Mandi M.L. Wild	Disrupting ER quality control alleviates glycosylation stress in a *Drosophila* model of the rare disease, DPAGT1-CDG.
45	Mark Yorio	ptpn-22 Mutations Affect Neuronal Migration and Axon Guidance in *Caenorhabditis elegans*.

A Special Thanks to Our Sponsors

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 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:

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

KU THE UNIVERSITY OF KANSAS
Center for Molecular Analysis of Disease Pathways

Enabling technologies are critical for the understanding of the biochemical and physical basis of disease as well as its diagnosis. The COBRE CMADP pursues health-related research that is focused on the development and application of these technologies to understanding disease processes. Two of the Center's major missions are to recruit, support and mentor outstanding junior faculty investigators, and to establish, operate, and grow successful Core Labs that provide state-of-the-art capabilities and services to investigators.

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.

MB GSO
Molecular Biosciences Graduate Student Organization

K·INBRE
Kansas IDeA Network of Biomedical Research Excellence

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

Poster Abstracts

1. Autoimmunity-associated allele of Ptpn22 distinctly modulates different toll-like receptor responses in bone marrow derived macrophages. **Tatum P. Aikin**

   During disease, the macrophage, a type of innate immune cell, carries out a variety of roles crucial to mounting an effective immune response. A major function of macrophages is recognition of pathogens via pattern recognition receptors (PRRs), such as the toll-like receptor family (TLRs), leading to inflammation and immune initiation. Regulation of functions such as this is important in maintaining homeostasis, balancing protectivity against pathogens and prevention of tissue damage caused by immune overactivity. Allelic differences in the genes that regulate immunity can disrupt this balance, impacting disease outcomes. Protein Tyrosine Phosphatase Non-Receptor Type 22 (Ptpn22) encodes the phosphatase Lyp (PEP in mice), regulating immune cell function. An alternative allele, 1858C>T, is common in the North American population and has been linked to a higher risk of autoimmunity. Previously, our group showed that mice carrying the alternative allele (PEP-R619W) exhibited an enhanced anti-viral and anti-tumor immune response relative to the major (wild-type) allele. However, the molecular mechanisms responsible for this enhanced response are incompletely defined, and a significant gap remains in understanding how PEP-R619W impacts macrophage biology. We hypothesize that PEP-R619W changes macrophage responsiveness to different TLR stimuli due to differences in downstream signaling. We test this by exposing wild-type (PEP-WT) and PEP-R619W bone marrow derived macrophages (BMMs) to various agonists, stimulating distinct receptors. ELISA and flow cytometry analysis revealed that PEP-R619W macrophages have increased functionality in response to TLR-3 and TLR-4 stimulation. These studies give insight into how this allele may be impacting macrophage biology and disease pathology.

2. Hypoxia tolerance in natural high-altitude populations of *Drosophila melanogaster*. **Lane Anaya**

   High altitude environments cause stress on local populations due to low temperatures, increased UV exposure, hypoxia (low oxygen), and aridity. Hypoxic conditions can cause elevated levels of inflammation, abnormal neuronal development, and sensory perception issues leading to physiological abnormalities and genomic adaptations. Previous work has suggested a link between high-altitude and sensory receptor genes

and related tissues, in vertebrates (i.e mammals), though whether this pattern applies to invertebrates is unknown. Therefore, I will be investigating hypoxia tolerance in high and low altitude wild populations of *Drosophila melanogaster*, paying close attention to sensory genes, like odorant/ionotropic/gustatory receptors. First, I compared three high altitude populations (~2,700m) to four low altitude populations (~500m) of wild-caught Ethiopian *D. melanogaster*. Each was tested for tolerance by subjection to three hours of near-anoxia and were then assessed for survival, negative geotaxis, and fecundity. Analyses revealed that high altitude populations were significantly more resilient to acute hypoxia exposure, with the signal being driven almost completely by females ($p<0.0001$). Second, I compared transcriptional differences between the same populations in head-only tissue. Gene ontology results showed downregulation of genes associated with 'sensory perception of smell', 'sensory perception of chemical stimulus', 'response to heat', and 'immune system processes'. These findings suggest a potential link between increased hypoxia tolerance and downregulation of chemosensory gene expression in high altitude populations of *D. melanogaster*. Additional testing of hypoxia tolerance between the two populations will utilize microrespirometry to measure oxygen consumption as a metric of relative metabolic rate.

3. The Autoimmunity-Associated Minor Allele of PTPN22 Accelerates NK Cell Development and Augments Effector Function. **Alec M. Bevis**

   Ptpn22 is expressed exclusively in immune cells and shows the highest expression in natural killer (NK) cells, yet its role in NK cells is unknown. Mice expressing the autoimmunity-associated minor allele of Ptpn22, PEP-R619W, have enhanced protection and antiviral immunity during murine coronavirus infection. This protective phenotype is partially driven by augmenting NK cell effector function to restrict viral replication. Due to these findings, we wanted to define the role of Ptpn22 and its PEP-R619W variant in NK cells during development and how they differentially regulate cytokine-mediated activation and cytotoxic function. To investigate the role of Ptpn22 and its variant in NK cell biology, we utilized wild-type (PEP-WT) and CRISPR-Cas9-generated Ptpn22 knockout (PEP-null) and PEP-R619W mice. We assessed the impact of PEP-null and PEP-R619W on NK cell development, maturation, cytokine signaling, and cytotoxic function using flow cytometry. In the bone marrow, NK cell numbers were increased, and maturation was accelerated in PEP-null and, more so, in PEP-R619W mice compared to PEP-WT. Mechanistically, Ptpn22 restricts IL-15 signaling, as PEP-null and PEP-R619W NK cells showed enhanced Granzyme B and pro-survival protein expression (Ki67, Bcl-2) following IL-15 stimulation. Furthermore, the addition of IL-15 to IL-12 and IL-18 stimulation elevated IFN-γ production in PEP-R619W NK cells over PEP-WT and PEP-null NK cells. Functionally, PEP-null and

PEP-R619W NK cells displayed superior cytotoxicity against tumor targets ex vivo, with the R619W variant again being the most potent. Our findings reveal that Ptpn22 is a novel regulator of IL-15 signaling and that its PEP-R619W variant modulates this pathway through additional mechanisms leading to heightened IL-15 responsiveness. This leads to NK cells with augmented effector functions compared to PEP-WT and PEP-null, highlighting the pleiotropic effects of this autoimmune-associated allelic variant.

4. The RNA-binding protein HuR regulates CD147 glycosylation 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. The RNA-binding protein HuR is overexpressed in TNBC and stabilizes oncogenic mRNAs, contributing to tumor growth and treatment resistance. CD147 (EMMPRIN), a transmembrane glycoprotein drives cancer hallmarks not limited to cellular invasion, migration, immune evasion and treatment resistance. HuR binds to the 3′-UTR of CD147 mRNA, enhancing its stability and protein expression. Highly glycosylated CD147 isoforms are expressed in multiple cancers, including TNBC, and their expression is associated with tumor progression and immune evasion. Our preliminary data indicates that HuR knockout reduces CD147 proteins expressed on tumor cells. We identified small molecule inhibitors (KH compounds) that disrupt HuR-mRNA interactions. Treatment of wild type TNBC cells with the KH compound has been reported to reduce CD147 proteins levels on these cells. However, the effect of HuR inhibition on CD147 glycosylation in TNBC is not well understood. In this study we aim to investigate the biological function of HuR in regulating the glycosyl forms of CD147 protein in TNBC and further determine whether HuR-mediated regulation of CD147 glycosylation influences the sensitivity of TNBC to immunotherapy.

5. Computational Investigation of Import Mechanisms of Bacterial Toxin Colicin E1 Through the Outer Membrane of *E. coli*. **Justin Carbone**

Colicins are bacterial warfare proteins encoded to destroy surrounding bacterial cells. Colicin E1 is specifically designed to perform RNAse function after crossing the *E. coli* outer membrane. Previously, Colicin E1 was crystalized embedded within TolC, but the molecular mechanisms that drive its membrane engagement and translocation remain incompletely understood. Here, we used molecular dynamics, constant-pH molecular dynamics, charged membrane-mimetic simulations, and normal mode analysis to investigate how protonation, intrinsic flexibility, and external electrostatic

interactions influence Colicin E1 conformational dynamics. Simulations revealed a highly flexible hinge centered near Pro110 and distinct electrostatic preferences between the T-domain and receptor-binding domain, with the T-domain preferentially interacting with positively charged surfaces while the receptor-binding domain favored negatively charged surfaces. Together, these results support a mechanism in which spatially heterogeneous electrostatic interactions with the outer membrane promote domain-specific membrane engagement and conformational rearrangement of Colicin E1, providing a potential pathway for toxin translocation into the periplasm.

6. Haspin Regulates the Checkpoint Monitoring TopoII Decatenation Activity to Maintain Chromosome Stability. **Asbin Chand**

   Chromosome segregation errors lead to chromosome instability (CIN), a hallmark of many cancers. DNA topoisomerase II (TopoII) is an essential enzyme that regulates chromosome decatenation and segregation by catalyzing a unique strand passage reaction. Perturbation of this enzymatic cycle can result in genomic instability; however, the mechanisms that monitor TopoII activity during this intricate enzyme cycle to ensure faithful chromosome segregation remain poorly understood. We previously showed that inhibition of TopoII with its catalytic inhibitor, ICRF-193, induces a non-canonical metaphase arrest known as the TopoII Responsive Checkpoint (TRC). Furthermore, inhibition of the mitotic kinases Haspin or Aurora B completely abrogates TRC activation, suggesting that these kinases are required for checkpoint function. Haspin phosphorylates histone H3 at threonine 3 (H3T3p) during mitosis, creating a docking site for the chromosomal passenger complex, which contains Aurora B as its catalytic subunit. In this study, we investigated the role of the Haspin-H3T3p-Aurora B signaling axis in the TRC. We found that activation of the TRC by ICRF-193 redistributes H3T3 phosphorylation on mitotic chromosomes. In addition, H3S28 phosphorylation, an Aurora B substrate, is Haspin-dependent and exhibits a localization pattern similar to that of H3T3p following TRC activation. Together, these findings suggest the Haspin-H3T3p-Aurora B signaling axis as a key component of the TopoII Responsive Checkpoint and indicate that it contributes to the surveillance mechanisms that preserve chromosome segregation during mitosis.

7. PTPN22 Alternative Allele Changes the Mechanisms of T Cell Mediated Weight Loss. **Mollie Coffey**

   Immune cells can drive pathology, including weight loss, during virus infection. Specifically, CD8 T cells drive cachexia in C57BL/6 wildtype mice during chronic virus infection of LCMV-cl13. CD8 T cells are necessary to clear LCMV-cl13 infection.

Previously, we demonstrated that mice expressing an autoimmune allelic variant in PTPN22 (PEP-R619W) experience transient weight loss, but can clear LCMV-cl13. As expected, when CD8 T cells were not present in PEP-WT mice, no weight loss occurred and had high viral titer. Surprisingly, PEP-R619W mice lost a significant amount of total body weight, specifically in adipose tissue weight when CD8 T cells or all T cells were depleted. We hypothesized that CD8 T cells in PEP-R619W mice were driving viral clearance and weight loss. To test this, we depleted CD8 T cells, or all T cells, in PEP-WT and PEP-R619W mice during LCMV-cl13 infection. We also infected T and B cell deficient Rag1KO PEP-WT and Rag1KO PEP-R619W mice. Tracking their weight loss, determined adipose tissue weight, and quantified the amount of virus titer. Rag1KO PEP-R619W mice did not lose weight. PEP-R619W results suggest that the mutation is acting in an unexpected way to drive transient weight loss during virus infection.

8. Elucidating Colicin E1 Mechanism of Action. **Andres Cordova**

Colicins are plasmid-encoded antimicrobial proteins produced by, and lethal to *Escherichia coli*. Their tripartite architecture of receptor-binding (R), translocation (T), and cytotoxic (C) domains mediate outer membrane protein (OMP) recognition, import, and killing, respectively. Colicin E1 (ColE1) is a pore-forming, group A colicin that uses BtuB and TolC for entry. Previous work from the Slusky lab showed that ColE1 fragments lacking the C domain remain stably bound to TolC; contrasting with other characterized group A colicins that fully traverse the outer membrane. Although the C domain ultimately inserts into the inner membrane, whether full-length ColE1 completely translocates remains unresolved. Elucidating this mechanism could enable the lab to design novel proteins/peptides targeting accessible *E. coli* OMPs: if ColE1 remains bound to the cell surface, the regions that interact with TolC could be used to obstruct antibiotic efflux; whereas, if it translocates, its sequence could instead be used to develop targeted drug-delivery proteins. To investigate this, we are collaborating with the Biteen lab (University of Michigan) to observe the position of each ColE1 domain by live-cell single-molecule fluorescence tracking. We generated cysteine-labeling mutants in the T (S185C), R (S316C), and C (523C, C-terminus) domains. Spot-killing assays against wild-type BW25113, ΔBtuB, and ΔTolC indicator strains showed no significant change in cytotoxic activity compared to wild-type, suggesting that removing the native cysteine (C505S), introducing domain-specific cysteine substitutions, and labeling with sulfo-Cy3 or sulfo-Cy5 maleimide did not significantly alter ColE1's activity. These validated fluorescent constructs will enable further analysis of ColE1 localization and translocation to investigate its entry pathway.

9. Anti-virulence Activity of Host Inflammatory Cytokines IL-1β and IFN-γ Against *Salmonella enterica.* **Larissa da Silva Ferreira**

   Enteric infections are a major global health concern, aggravated by the increasing incidence of antimicrobial resistance. *Salmonella enterica*, a foodborne pathogen, possesses the ability to cause severe disease in immunocompromised individuals, attributed to major virulence factors contributing to environmental persistence, host colonization, and increased tolerance to antimicrobial stress. Despite extensive research of its virulence mechanisms and how it interacts with the host, how the pathogen senses and responds to signals produced by the host immune system remains unclear. Cytokines have been reported to influence virulence in several bacterial species; however, their direct influence on *Salmonella enterica* virulence remains poorly characterized. The objective of this study was to investigate the effects of the pro-inflammatory cytokines IL-1β and IFN-γ on S. enterica virulence-associated traits. Biofilm production was quantified using a crystal violet microtiter plate assay following cytokine exposure. Planktonic growth was also evaluated independently. Both assays used 96-well microtiter plate assays and a microplate reader (SpectraMax, Molecular Devices). Bacterial motility was assessed by directly observing bacterial cells swimming under the microscope and analyzed using ImageJ software to determine whether cytokine exposure altered motility-associated phenotypes. To further characterize cytokine activity, heat-inactivated IL-1β and IFN-γ were also evaluated for their effects on biofilm formation. Both IL-1β and IFN-γ significantly reduced biofilm formation while increasing bacterial motility without affecting bacterial growth, indicating that these phenotypes were independent of changes in proliferation. Heat inactivation at 90℃ for 30 minutes abolished the inhibitory activity of IL-1β, restoring biofilm formation to control levels, whereas IFN-γ retained inhibitory activity and exhibited only partial recovery after prolonged heat treatment for one hour. These findings demonstrate that host inflammatory cytokines can directly alter S. enterica biofilm formation and suggest that the biofilm-modulating activities of IL-1β and IFN-γ differ in their sensitivity to heat treatment. Understanding how inflammatory cytokines influence bacterial behavior provides new insight into host-pathogen communication and may reveal mechanisms by which inflammatory environments are evaded while shaping the behavior of *Salmonella enterica* during infection.

10. Role of C-Terminal Domain Phosphorylation in Topoisomerase IIα During Mitosis. **Ayushee Dasgupta**

    DNA Topoisomerase IIα is an essential enzyme that resolves the DNA's topological stress during mitosis to ensure faithful chromosome segregation. While the catalytic core of Topo IIα is well studied, the intrinsically disordered C-terminal domain (CTD,

amino acid position from 1215 to 1531) is less understood, but it has essential role in mitosis, controlled by its post-translational modification, such as phosphorylation and SUMOylation. Mitotic phosphorylation of CTD, including the MPM2 (Mitotic Protein Monoclonal #2) site at S1469 and at S1524, is thought to regulate Topo IIα for its essential role in chromosome segregation remain unclear. This study examines the function of the conserved phosphorylation sites within the CTD by creating phospho-deficient (Serine à Alanine) and phospho-mimic (Serine à Aspartate) mutants of human Topo IIα at residues S1469 and S1524. These mutant constructs were created using site-directed mutagenesis and expressed in mammalian cells using an auxin-inducible degron (AID) and Tet-On expression system, allowing endogenous Topo IIα to be degraded while inducing expression of mutant Topo IIα. Protein expression and chromosome association are being analyzed by western blot and chromosome fractionation to determine how phosphorylation at these sites affects Topo IIα localization during mitosis.

11. Catanionic Detergent Vesicles as a Model Linking Protomembranes to Modern Membranes. **Andrew Daufel**

    Membranes are the physical barriers life uses to control the flow of small molecules into and out of the cell. This control is necessary to keep the cell alive, and the membrane defines the boundaries of the self. How did the membrane come to be? Modern membranes are lipid bilayers containing amphipathic molecules with multiple hydrophobic tails. However, because of the chemical complexity of multitailed amphiphiles, spontaneous synthesis of such amphiphiles in quantities needed for a membrane is unlikely. In contrast, bilayers can also be formed by more simplistic single-tailed amphiphiles via catanionic detergent vesicles (CDVs). Here we demonstrate that CDVs are protein competent, a necessary component of a protobilayer. We find CDVs are also highly thermostable, form at physiological pHs, and are resistant to ionic strength. Given these properties we see CDVs as a possible answer to what the protomembrane of LUCA may have looked like. CDVs can also be used in modern applications, as unlike other synthetic bilayers, CDVs are charge-asymmetric akin to modern membranes. Using this property, we use CDVs to reconstitute outer membrane proteins (OMPs) in a bilayer resembling the strict charge asymmetry of the outer membrane (OM). Finally, CDVs have potential applications as a mimetic for proteins which are difficult to reconstituting in other membrane mimetics.

12. Mapping autosomal supression to meiotic drive. **Tessa K. Eads**

    Selfish genetic elements are single genes or gene complexes that cheat mendelian segregation and bias their way into the next generation. In the *Drosophila affinis*

species, a gene complex on the X chromosome prevents the proper development of Y-bearing sperm, resulting in female-biased progeny. This is also referred to as sex-ratio meiotic drive. As the transmission of the driving X chromosome increases, there is strong selection on autosomal loci to suppress the X-linked driver. In this study, we will use a QTL mapping approach to identify specific regions of the genome that influence suppression. Following the mapping experiment, we will perform RNAseq to observe the differentially expressed genes that are involved in autosomal suppression.

13. Capturing the host ADP-ribosylome to identify host factors that restrict coronavirus replication. **Piero Rodolfo Espinel Cabrera**

    All coronaviruses (CoV) encode a conserved Macrodomain (Mac1) within nonstructural protein 3 (nsp3) that erases (hydrolase activity) mono-ADP-ribose (ADPr) on acidic residues and reads ADPr moieties at non-acidic residues. Mac1 is a key virulence factor to promote CoV replication and pathogenesis with the biological function to counter host immune defenses modulated by the activity of PARP(s) that deposit ADPr on target proteins. Using reverse genetics we have identified mutations that separately reduce the reader and eraser activity of Mac1, and these mutant viruses have demonstrated that both functions are critical for viral replication. However, the ADP-ribosylated host factors and in some cases even the PARP enzymes that restrict CoV infection remain unknown. Our immediate goal is to capture the global ADPr proteome following CoV infection and assess species-specific host factors that suppress CoV replication. To identify ADP-ribosylated proteins we have cloned GFP-tagged archaeal macrodomain, called MacroGreen, optimized to have high affinity for ADPr into a eukaryotic expression construct. MacroGreen specifically pulls down ADP-ribosylated proteins in both naïve and murine hepatitis virus (MHV) infected cells and does not and does not impact MHV replication. Furthermore, initial Co-IP mass spec experiments have observed an enrichment of several auto-ADP-ribosylated PARP proteins (PARP1, PARP5a, PARP8, and PARP3), validating this method. Long-term, our goal is to utilize MacroGreen to identify ADP-ribosylated proteins that impact CoV infection and other important cellular functions. MacroGreen will allow us to identify ADP-ribosylated proteins following multiple viral perturbations and tease out intrinsic, species-specific host factors involved in restricting or promoting virus replication.

14. Diversification of heterochromatin genes across polyploid *Xenopus* species. **Cael Gorman**

    Whole-genome duplication profoundly influences genome evolution by generating additional gene copies that may undergo subfunctionalization, neofunctionalization, or

long-term conservation. *Xenopus* frogs provide a valuable system for studying these processes, as duplication events have produced multiple polyploid species. This study investigates protein sequences encoded by 13 candidate genes involved in the establishment and maintenance of constitutive heterochromatin in *X. tropicalis* (diploid), *X. laevis* (tetraploid), *X. boumbaensis* (octoploid), and *X. longipes* (dodecaploid). Because constitutive heterochromatin is essential for maintaining genome stability and chromosome integrity, comparing sequence variation in these genes across species may provide insight into how their functions have been maintained or diversified following polyploidization. Protein sequences for *X. tropicalis* and *X. laevis* were obtained from NCBI, while sequences for *X. boumbaensis* and *X. longipes* are unpublished and obtained from newly generated transcriptomes. Candidate genes were identified using BLAST, aligned, and analyzed using pairwise protein sequence comparisons after removing redundant isoforms. Across candidate genes, *X. tropicalis* exhibited the highest overall mean species-level protein sequence identity (96.17%), whereas *X. laevis* exhibited the lowest (91.22%). Candidate genes were then grouped into four functional categories: Heterochromatin Protein 1 (HP1), DNA methyltransferases, histone demethylases, and histone methyltransferases. HP1 genes exhibited the highest mean cross-species sequence identity (96.10%), whereas histone methyltransferases exhibited the lowest (89.56%). These preliminary findings provide a foundation for future analyses integrating transcript isoform diversity and additional protein sequence variation to better understand how whole-genome duplication has shaped genes involved in constitutive heterochromatin maintenance.

15. University of Kansas Nanofabrication Facility: Equipment and Capabilities. **Haider Warraich**

The University of Kansas 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 also accommodate broad research applications with micro- and nanofabrication needs. The facility 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, thin film deposition, scanning electron microscopy (VP-SEM), atomic force microscopy, contact angle goniometry, ellipsometry, profilometry, wafer dicing, wire bonding, laser ablation and engraving, 3D printing, hot embossing, and COMSOL software for device modeling. In addition, the facility has numerous microscopes for general inspection, ovens and furnaces, ultrapure water, and dedicated process fume hoods. This facility is under the leadership of Dr. Susan Lunte,

and the direction of Ryan Grigsby. Services and usage of the facility are available to researchers from all Kansas universities. Training is provided to new investigators and graduate students in the use of micro- and nanofabrication procedures and equipment. In addition, researchers from both non-Kansas academic and private industry institutions may contract with the facility for consultation and services, including access to the facility. Hourly and per-use rates apply for facility access, equipment usage, and staff labor. Consultation is free.

16. Genome Sequencing Core – KU-Lawrence. **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/.

17. Pyroglutamic Acid Contributes to the Anti-Virulence Activity of *Staphylococcus epidermidis* Against *Staphylococcus aureus.* **Kiana Hajiarbabi**

    Staphylococcus aureus is one of the world's most problematic bacterial pathogens due to its widespread antibiotic resistance and its ability to form biofilms, which protect the bacteria from both host immune defenses and antimicrobial therapies. The skin microbiome contains commensal bacteria that produce compounds capable of inhibiting *S. aureus* and other pathogens. Our lab previously showed that one of the most abundant skin commensals, *Staphylococcus epidermidis*, produces secreted compounds that reduce *S. aureus* biofilm formation without affecting bacterial growth. Recently, we observed that these compounds also inhibit *S. aureus* adhesion to and

invasion of keratinocytes and reduce biofilm formation under shear flow conditions that better mimic the in vivo environment. Purification of the active fraction revealed that pyroglutamic acid is one of the antibiofilm compounds present in the *S. epidermidis* supernatant. However, pyroglutamic acid alone did not reduce *S. aureus* biofilm formation under shear flow conditions or inhibit adhesion to and invasion of keratinocytes, suggesting that additional bioactive compounds are responsible for these activities. Treatment with pyroglutamic acid in a murine skin infection model significantly reduced *S. aureus* dissemination to the kidneys and spleen, although no reduction was observed at the primary skin infection site. Overall, these findings suggest that pyroglutamic acid contributes to the biological activity of the *S. epidermidis* supernatant but does not account for all of its observed anti-virulence effects. The reduction in systemic bacterial burden observed in vivo highlights pyroglutamic acid as a promising candidate for further investigation as an antivirulence compound against *S. aureus* infections.

18. Three-Dimensional Quantification of Heterochromatin Domain Organization During Early *Xenopus* Development. **Danny Jack**

    Heterochromatin is an important part of the genome that helps regulate gene expression and maintain genome stability during development. However, how heterochromatin changes during early embryonic development is not fully understood. In this study, early Xenopus embryos were stained for H3K9me3, histone H3, and DNA and imaged using fluorescence microscopy. Images were analyzed using an application called “Imaris” to create three-dimensional models of nuclei and heterochromatin domains. Domain number, volume, surface area, and fluorescence intensity were measured and compared across developmental stages. The results showed that heterochromatin organization changes as embryos develop, suggesting that chromatin is reorganized during early embryogenesis. Using Imaris allowed for consistent and accurate measurement of these changes in three dimensions. This study demonstrates that quantitative image analysis is a useful approach for studying heterochromatin organization and provides a foundation for future research on how chromatin structure changes during early development.

19. Antibiotic selection shapes quorum sensing evolution through adaptive mutations in *Pseudomonas aeruginosa.* **Sumaya A. Jahin**

    Pseudomonas aeruginosa is a multidrug-resistant opportunistic pathogen and a model organism for studying quorum sensing (QS), a density-dependent signaling system in which LasI produces the signal 3OC12-HSL and LasR acts as its transcriptional receptor and regulator. QS regulates virulence and antibiotic resistance,

making it a promising therapeutic target. Paradoxically, however, lasR loss-of-function mutants are frequently isolated from chronic infections, suggesting that adaptive mutations under antibiotic selection favor their emergence. We hypothesized that adaptive mutations facilitate lasR mutant emergence and persistence by enabling LasR-independent survival under antibiotic selection. Previously, using experimental evolution, we identified a mutation in fusA1, encoding elongation factor EF-G1A, that specifically increased tobramycin resistance in lasR mutants. To determine whether similar evolutionary mechanisms operate during gentamicin adaptation, we performed long-term evolution experiments with *P. aeruginosa* PA14 in casein medium under no antibiotic, fixed sub-MIC gentamicin (0.5 μg/mL), or gradually increasing gentamicin concentrations (0.5–5.7 μg/mL). In antibiotic-free populations, lasR mutants first became detectable within 4–8 days, whereas gentamicin delayed their emergence to 8–28 days, with the greatest delay observed under increasing gentamicin selection. Once detectable, lasR mutants rapidly expanded and dominated most populations. Whole-genome sequencing of representative LasR+ and LasR- isolates from one focal population identified shared mutations in ptsP and gdhB, while the LasR- isolate carried a chromosomal inversion disrupting both lasR and fliK, and the LasR+ isolate harbored a unique mutation in pmrB. The evolved LasR+ isolate exhibited approximately 6-fold higher gentamicin MIC than LasR- isolates obtained from the same population, yet the highly resistant LasR+ variant failed to proliferate and LasR- variants continued to dominate the population. These findings suggest that multiple adaptive mutations along with population dynamics shape QS evolution under antibiotic selection.

20. Ultra-High-Throughput Virtual Screening and Binding Site Prediction. **Andrew M. 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 and Molecular Modeling Core. **David K Johnson**

    Part of the Chemical Biology of Infectious Disease COBRE at the University of Kansas, the Computational Chemical Biology and Molecular Modeling Core (CCBMM) provides the computational resources and expertise to enhance the productivity of researchers studying infectious diseases, in addition to other projects. The CCBMM 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 CCBMM. 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 CCBMM 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. Flow Cytometry Core: A Chemical Biology of Infectious Disease COBRE Core Laboratory. **Peter R. 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.

23. Investigating the Genetic Impact of Flock House Virus in *Drosophila melanogaster.* **Alexis M. Michalski**

Flock House Virus (FHV) is a non-enveloped positive-sense single-stranded RNA (ssRNA) virus that readily infects Drosophila cell lines, including DL-1 and S2 cells. FHV is unique because it possesses one of the smallest known genomes among animal RNA viruses and is capable of replicating in diverse cell types, including insect, plant, mammalian, and yeast cells. Contrary to many other viruses, FHV has been extensively studied in vitro, but lacks in vivo studies. This work aims to identify host genetic factors that influence antiviral immunity, expanding our understanding of host-virus interactions between FHV and *Drosophila melanogaster* while also providing insight into possibly conserved mechanisms of antiviral immunity across species. To address this gap, we use extreme quantitative trait locus (X-QTL) mapping in a multiparental recombinant *Drosophila melanogaster* population founded from eight inbred lines, enabling high-resolution mapping of complex traits. Here, multiple replicated sets of male *Drosophila melanogaster* from the multiparental recombinant population are infected with FHV. From each replicate, we select the final 20% of animals remaining alive (the most resistant individuals), along with a set of uninfected controls. Pooled whole-genome sequencing is then performed on both control and resistant groups, followed by QTL mapping to identify genomic regions that display allele frequency shifts between the experimental and control pools. Previously, we have completed an experimental replicate that sampled approximately 3K flies. Currently, we are generating additional larger experimental replicates to increase mapping power and QTL resolution. Once completed, we will identify candidate genes within significant QTL peaks using FlyBase. These candidate genes, in addition to well-known immune genes, will be validated to determine their contributions to antiviral resistance and to investigate their roles within immune pathways.

24. Host-derived Interferon-gamma modulates *Salmonella enterica* virulence and host cell interactions. **Ella Miller**

Bacterial pathogens continuously adapt to the host environment by sensing environmental and host-derived signals. While hormones, neurotransmitters, and cytokines have been shown to modulate virulence in several bacterial species, whether inflammatory cytokines directly regulate *Salmonella enterica* virulence remains poorly understood. Given the central role of the HilA regulator in controlling the expression of genes required for host epithelial cell invasion, we investigated whether exposure to the pro-inflammatory cytokine IFN-γ modulates hilA expression and alters bacterial interactions with epithelial cells and macrophages. *Salmonella* was cultured for 4 hours in the presence of IFN-γ or BSA, and hilA expression was evaluated by flow cytometry using a reporter strain containing the promoter of hilA fused to GFP, as well as by quantitative Real-Time PCR (qPCR). The impact of IFN-γ exposure on bacterial infection was subsequently assessed in human colonic epithelial cells (HT-29) and murine macrophages (RAW 264.7). Intracellular bacterial colonization was quantified using colony-forming unit (CFU) assays, in which host cells were lysed following infection and viable bacteria were enumerated by serial dilution and plating. IFN-γ significantly increased hilA expression by both flow cytometry and qPCR approaches. Contrary to these findings, however, *Salmonella* subcultured in the presence of IFN-γ exhibited reduced invasion of HT-29 cells and reduced colonization of RAW 264.7 macrophages at 2 h post-infection. Together, these findings demonstrate that IFN-γ modulates *Salmonella* virulence-associated gene expression but does not necessarily enhance host cell invasion or colonization. These results highlight the complex role of inflammatory cytokines in host-pathogen interactions and microbial adaptation during infection.

25. Contribution of PEP-null and PEP-R619W Antigen-Presenting Cells in enhancing CD4 T cell function during chronic viral infections. **Ishita Mirchandani**

Chronic viral infections promote a state of immune dysfunction called T cell exhaustion. T cell exhaustion is characterized by reduced cytokine production, reduced cell proliferation, and failure to form immune memory. CD4 T cell exhaustion is of particular concern as CD4 T cells are necessary for antiviral CD8 T cell response and B cell antibody production. Prevention of CD4 T cell exhaustion enhances antiviral immune response; however, the mechanisms underlying the prevention or reversal of CD4 T cell exhaustion during viral infection is not known. Protein Tyrosine Phosphatase Non-Receptor Type 22 (PTPN22) was recently shown to promote CD4 T cell exhaustion. Mice which lack PTPN22 (PEP-null) or have a common allelic variant (PEP-R619W) have enhanced antiviral CD4 T cell function and accelerated LCMV-cl13 infection clearance. Both CD4 T cell intrinsic and CD4 T cell extrinsic mechanisms contribute to enhanced CD4 T cell function. Here, we test the hypothesis

that PEP-R619W and PEP-null Dendritic cells (DCs) are more immunostimulatory, which promotes CD4 T cell function and reduces exhaustion. To test this, we employ a PTPN22 CD11c conditional knockout mouse (cKO) and validate a novel mouse model where we can Switch the PEP-wildtype Allele to R619W via the Cre Lox system (the “SPARCL” mouse). Using our PTPN22 cKO mouse we found that during chronic LCMV-cl13 infection PEP-KO and CD11c cKO have better survival then PEP-WT. Through DC–CD4 T cell coculture we found that PEP-R619W CD4 T cells with PEP-R619W DCs in coculture are producing higher amount of IL-2 and interferon gamma as compared to PEP-WT. These findings suggest that PTPN22 regulates DC mediated CD4 T cell function during viral infection. To investigate the cell type specific effects of PEP-R619W allele in vivo, we are currently validating the SPARCL mouse model. As an initial step in this process, we are purifying Cre recombinase protein. These findings provide foundation for in vivo studies to identify which Antigen-Presenting Cells and mechanism is promoting CD4 T cell function in PEP-R619W and PEP-null mice.

26. Identification and analysis of repurposed therapeutics for the rare mitochondrial disease, WARS2 deficiency, using a *Drosophila melanogaster* model. **Tymber Moody**

    Patients with WARS2 Deficiency face severe neurological symptoms, including seizures and movement defects, with no curative treatment available. WARS2 encodes mitochondrial tryptophanyl-tRNA synthetase, which charges mitochondrial tRNA with tryptophan, an essential step in mitochondrial protein synthesis. Our goal was to identify drugs that improve disease-associated phenotypes. To model this disorder, we knocked down WARS2 expression in the eye of *Drosophila melanogaster*, producing a small, rough-eye phenotype that enabled quantitative measurement of eye area. We then screened a library of 1,520 repurposed drugs, an approach that uses existing FDA/EMA-approved drugs in a new context to accelerate the translation of treatments to patients. We classified drugs as positive hits if they rescued the phenotype, defined as increased eye area relative to untreated controls. The screen yielded a 4.8% hit rate, with antibiotics emerging as the top-performing drug class. To test whether these findings extend to behavioral phenotypes, we generated a movement model by knocking down WARS2 in muscle, raising flies on drug-containing food and evaluating climbing ability with a negative geotaxis assay. One antibiotic improved movement phenotypes, consistent with the eye model, suggesting this drug is not tissue-specific and can rescue multiple patient-modeled symptoms. This same antibiotic also rescued the eye phenotype in a model of MARS2 Deficiency, a related tRNA synthetase disorder. Together, these findings show that drug repurposing in *Drosophila melanogaster* can identify compounds that rescue both

morphological and behavioral phenotypes associated with WARS2 deficiency, and that select antibiotics may be promising therapeutic candidates for tRNA synthetase-related mitochondrial disorders.

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

    As both a gut commensal and opportunistic pathogen, *Enterococcus faecalis* relies on a broad range of carbon substrates for metabolism, including host-derived glycans when preferred carbon sources are scarce. We identified a six-gene operon, encoding an ABC transporter and an associated signal transduction system, that is induced in response to low glucose and the presence of high-mannose N-linked glycans. Using luciferase reporter assays, we show that operon expression depends on a functional YesLMN signaling pathway. We constructed mutants of each gene in the pathway and complemented those mutations with either the native gene or site-specific mutations predicted to alter the signaling pathway and examined their contributions to the activation of operon expression using an ef2223 promoter -luciferase fusion. We show that the kinase activity of YesM is critical for phospho-signaling to YesN and that the associated phosphatase activity of YesM suppresses signaling in the absence of host ligands, when YesN can be phosphorylated in a YesM independent manner presumably through cellular acetyl phosphate pools. To determine whether YesN contributes to pathogenesis, we compared wild-type and yesN mutant strains in a murine catheter-associated urinary tract infection model, and found that the yesN mutant was impaired in its ability to disseminate from the bladder to distal sites, indicating that this glycan-sensing and import system supports *E. faecalis* virulence. To define the ligand specificity of the YesM sensor kinase, distinct high mannose ligands ranging from 1 mannose-GlcNAc moiety to 9 mannose-GlcNAc were chemically synthesized and tested for the ability to induce expression of the operon using the luciferase reporter. The results indicate that *E. faecalis* prefers the full-length high mannose 9-GlcNAc for optimal operon activation, and that this glycan ligand best supports growth of E. faecalis when these glycans are used as the principal carbon source. Future studies are being conducted to assess glycan ligand binding to the extracellular loop domain of YesM, as well as the ABC transporter substrate binding protein, EF2221 to determine glycan ligand affinity.

28. Gut microbiome-derived metabolites regulate *Vibrio cholerae* biofilm formation and host inflammatory responses. **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. Our study focuses on phylogenetically related gut commensals *Enterocloster citroniae* and *Clostridium innocuum* to uncover how these gut commensals influences pathogen behavior and host responses. Previous studies have shown that *E. citroniae* produces bioactive compounds that affect *Salmonella enterica* and *Vibrio cholerae* behavior. Here, we demonstrate that small molecules produced by *E. citroniae* significantly enhance biofilm formation, activate vpsR promoter, the master regulator of biofilm formation, and reduce swimming motility in *V. cholerae*. To identify the bioactive compounds inducing biofilm formation in *V. cholerae*, reversed-phase High-Performance Liquid Chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopy revealed indole as a major component of the *E. citroniae* extract, which is involved inducing biofilm formation in *V. cholerae*. We further show that this phenomenon is not limited to *E. citroniae*. *C. innocuum*, also produces bioactive compounds against *V. cholerae*. Interestingly, however, *C. innocuum* does not produce significant concentrations of indole, suggesting the presence of another bioactive compound. To investigate the effects of the such metabolites on host responses, we exposed LPS-stimulated murine macrophages (RAW 264.7) to metabolites produced by *E. citroniae* and *C. innocuum*. These metabolites modulated key inflammatory markers gene expression, indicating that small molecules produced by these gut commensals can directly influence host inflammatory responses. This study sheds light on the chemical biology of the gut microbiota and its ability to influence *V. cholerae* behavior, and host inflammatory responses, providing a foundation for future research into the interplay between pathogens, hosts, and the commensal microbiota.

29. Up-Regulating the cGAS-STING Pathway via HuR Inhibition in Cancer Cells. **Ngoc Huan Nguyen**

Cancer immunotherapies have achieved significant success compared to traditional methods including chemotherapy and radiotherapy; however, they face challenges such as low response rate. 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 is involved in various post-transcriptional regulatory processes, including mRNA splicing, maturation, nuclear export, stability, and translation. Consequently, HuR dysfunction contributes to the development of various diseases including cancers; and ubiquitous cytoplasmic HuR levels are found in various cancer types. Our lab has discovered HuR small molecule inhibitors (KHs) that inhibit the

HuR-mRNA interaction. We hypothesize that HuR promotes cancer immune evasion by suppressing the cGAS-STING pathway, HuR inhibition can overcome the immune-resistance and improve the response of cancer immunotherapies. This study will offer a promising strategy to improve cancer immunotherapy and further research is required to understand the mechanisms of how HuR regulates the cGAS-STING pathway, hence leads to the activation of immune system.

30. Toward Metal Binding Site Design Using a Protein Language Model. **David Noblett**

    Metal binding is an exceedingly important type of protein-ligand interaction, involved in nearly half of enzymatic reactions and usage in protein structural stability. Thus, designing metal binding sites is a potent method to develop novel activity in designed proteins. However, rational design of metal binding sites remains exceedingly difficult. Current methods often do not explicitly consider the role of supporting residues to a metal site or are focused most on de-novo design. The focus of this research is to develop a pipeline for metal binding site design that incorporates the broader protein context. To achieve this, we employ protein language model (PLMs) and graph neural networks (GNNs). The central hypothesis of this work – supported by preliminary evidence - is that PLM embeddings can distinguish metal coordinating residues from non-coordinating residues and exhibit a capacity to distinguish metal preference. By combining the two, we seek to uncover latent metal binding capacity in proteins. We first will develop a method to identify potential mutable sites in proteins that can become metal sites upon mutation. Second, we will develop a pipeline to explore the residues that drive metal specificity, and features that may allow metal site redesign. These methods together will provide insight into metalloproteins and step toward a more broadly applicable computational strategy for metal binding site design.

31. Evolution of resistance to *Candida auris* yeast among *Drosophila* species. **Lillian O'Donnell**

    Genes involved in innate immunity function at the interface of host-microbe interactions and are thus subject to strong coevolutionary pressures imposed by pathogens. Although immune responses vary substantially among closely related species, the genetic mechanisms sustaining this diversity remain poorly elucidated. Here, we used *Drosophila* flies as a model to identify loci underlying variation in resistance to *Candida auris*, an emerging multidrug-resistant yeast, infection. . First, we infected three closely related species of *Drosophila* (*D. simulans, D. sechellia, D. mauritiana*), all of which can produce viable hybrids, with *C. auris*. *D. simulans* exhibited high resistance to the pathogenic yeast (approximately 100% survival after 20 days), while *D. sechellia* was more susceptible (25-50% survival after 20 days).

We then generated F1 hybrids by crossing *D. simulans* females to *D. sechellia* males. F1 individuals were infected with *C. auris*, and we observed that hybrids displayed intermediate resistance between *D. simulans* and *D. sechellia*. We are currently generating backcross offspring by crossing F1 females to *D. sechellia* males, which will be used for *C. auris* infection assays and Quantitative Trait Locus mapping to identify genomic regions associated with yeast resistance. Candidate loci identified in our work will provide a foundation for investigating how evolutionary divergence in innate immune genes contributes to variation in resistance to yeast infection.

32. Metabolites produced by bacterial skin microbiome members disrupt *Staphylococcus aureus* biofilm formation. **Aidan O'Hara**

    The skin microbiome consists of bacteria, viruses, and eukaryotic microorganisms. Recent research has revealed that certain members of the skin microbiome can reduce the pathogenicity of bacterial skin pathogens, such as *Staphylococcus aureus*. One major virulence factor of *S. aureus* is its ability to produce biofilms. Biofilms confer enhanced resistance towards antimicrobials and the host's immune response to biofilm-producing bacteria, enabling hard-to-treat infections. Novel strategies must be developed to decrease the pathogenicity of *S. aureus*. The goal of this research was to identify specific bacterial skin microbiome members that produce metabolites that affect *S. aureus'* biofilm production. Species identification was performed using Matrix-Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry (MALDI-ToF MS). Metabolites were sourced from the cell-free conditioned media (CFCM) of bacterial skin isolates grown for 24 hours with aeration. These CFCMs were then assayed for their activity against *S. aureus* biofilm production or growth rate with a biofilm assay or growth curve assay, respectively. We have found that almost all *Staphylococcus epidermidis* strains tested instilled a significant reduction in *S. aureus'* biofilm production independent of growth. *Bacillus* spp. CFCM also showed an ability to decrease *S. aureus'* biofilm production. *Corynebacteria* spp. have variable activity against *S. aureus'* biofilm production, where some strains increase and others decrease production. Both phenotypes occur independent of changes in *S. aureus'* growth rate. The research presented showcases that the bacterial skin metabolome contains compounds that affect *S. aureus'* biofilm production in a growth-independent manner, highlighting the importance of the skin microbiome.

33. RNA-sequencing data of differential gene expression following HuR inhibition across multiple cancer cell lines. **Candice Osagie**

    This study presents an integrative transcriptomic analysis of KH-3, a small-molecule inhibitor targeting the RNA-binding protein HuR, across three human cancer cell lines:

HCT 116 (colorectal), MiaPaCa2 (pancreatic), and DU 145 (prostate). RNA-seq profiling and statistical modeling reveal hundreds of differentially expressed genes following KH-3 treatment, with a consistent signature of downregulated mRNAs across all lines. KEGG pathway enrichment highlights convergent disruption of cancer-associated pathways and RNA metabolism. RT-qPCR and RNA immunoprecipitation data confirm HuR-dependent gene regulation at the transcript and RNP complex level, validating the transcriptome findings. All raw data and processed results are shared as a FAIR²-compliant package, with transparent provenance and harmonized metadata, to support broad re-use and downstream hypothesis generation. This dataset provides a reproducible resource for understanding HuR-mediated post-transcriptional networks and characterizing the molecular impact of RNA-binding protein inhibition in diverse cancer models.

34. Functions of 3D Genome Organization. **Sarayu Pentapati**

Genomes are compartmentalized in order to regulate their function. For example the areas of the genome that are silenced are organized into heterochromatin domains, often found near the nucleolus and nuclear periphery. Within the early embryo, the genome becomes organized in 3D space during the mid-blastula transition. However, we still do not understand the processes that establish and maintain these structures in the early embryo. Using the African clawed frog *Xenopus laevis* as a model system, I fixed and stained different early stage embryo extracts for heterochromatin, DNA, and H3. I then quantified heterochromatin intensities across different stages of embryo extracts, and then plotted them with normalization to DNA. These results show that heterochromatin changes morphologically as well as in abundance throughout embryogenesis. This points towards potential factors and processes that may be responsible for these changes in heterochromatin establishment.

35. Musashi1 regulates growth of the mouse intestinal epithelium. **Bikash Pokhrel**

Colorectal cancer (CRC) is the second leading cause of cancer-related mortality, affecting approximately 1 in 24 individuals. CRC arises from disruptions in the balance of cell proliferation, differentiation, and apoptosis within the intestinal epithelium. Musashi1 (MSI1), an RNA-binding protein, is selectively expressed in stem and early progenitor cells and is essential for their maintenance and lineage decisions. Our previous work demonstrated that ubiquitous overexpression of Musashi1 in mice results in reduced overall organismal and organ growth. To elucidate the mechanism underlying this phenotype, we performed bulk RNA sequencing alongside complementary in-vitro assays. These studies revealed that Musashi1 suppresses growth by modulating the mTORC1 signaling pathway, a central regulator of cellular

growth. Immunofluorescence analyses of the intestine further showed that Musashi1 expression and mTORC1 activity are largely mutually exclusive at the cellular level, supporting a functional role for Musashi1 in regulating the fate and behavior of stem and dividing cells. Our ongoing work aims to identify the RNA targets through which Musashi1 controls mTORC1 signaling. Given that dysregulation of both Musashi1 and mTORC1 is a hallmark of colorectal cancer, these findings may provide insight into novel therapeutic strategies.

36. Intragenomic RNA-RNA interactions modulate 3′X RNA conformations in hepatitis C virus. **Ethan P. Rogers**

    The hepatitis C virus (HCV) genome is a positive-sense, single stranded RNA that contains several highly conserved structures that are critical for viral propagation. Here, we focus on two interacting RNA elements: 3′X and 5BSL3.2. Recent work has resolved the structural dynamics of 3′X, detailing its slow interconversion in bi-conformational equilibrium. In this presentation, we demonstrate how the 3′X conformational ensemble is impacted by 5BSL3.2 via single-molecule FRET and provide a new four-state model to describe this interaction. This approach allow us to quantitatively describe both the conformational equilibrium of 3′X and the binding affinities of the 3′X conformations for 5BSL3.2. Our results demonstrate that 5BSL3.2 readily associates with both conformations under a variety of conditions and indeed impacts the conformational equilibrium of 3′X, promoting one of its intrinsic conformations. These results support an emerging hypothesis that suggests the 3′X-5BSL3.2 interaction is central to a riboregulatory network that controls viral replication and translation.

37. The University of Kansas High Throughput Screening/Infectious Disease Assay Development Laboratory. **Anuradha Roy**

    The University of Kansas High Throughput Screening/Infectious Disease Assay Development Laboratory (KU-HTS/IDAD) is a fee-for-service, state-of-the-art facility dedicated to providing academia, nonprofit institutions, biotech, and pharmaceutical industries with exceptional assay development and high throughput screening 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. HTS-IDAD Lab has a collection of 400,000 small molecules that are available for screening campaigns. KU-HTS/IDAD lab is innovative and flexible in providing superior service to the drug discovery research community, including assay development, screening, compound profiling and data mining. The labs have capabilities of executing miniaturized assays using all available platform technologies

(except radiometric) in 384/1536-well microplates. KU-HTS/IDAD 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.

38. Neuronal Regulation of Pulmonary Endothelial Function During Carbapenem-Resistant *Klebsiella pneumoniae* Infection. **Bhawana Samal Magar**

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is a major cause of severe pneumonia and Gram-negative sepsis with limited treatment options due to multidrug resistance. CRKP-induced pneumonia is characterized by pulmonary inflammation and endothelial dysfunction, contributing to bacterial dissemination and poor clinical outcomes. Calcitonin gene-related peptide (CGRP), a neuropeptide released by nociceptive sensory neurons, is known to regulate neuroimmune interactions in infection and tissue inflammation. However, its role in pulmonary endothelial responses during CRKP infection is poorly understood. In our study, we used both in vitro and in vivo models to investigate the effects of CGRP on endothelial activation and barrier integrity. Human lung microvascular endothelial cells (HULEC-5a) were infected with CRKP in the presence or absence of recombinant CGRP (rCGRP). Endothelial barrier integrity was evaluated by immunofluorescence and transwell permeability assays, while gene expression of inflammatory and endothelial activation markers was assessed by quantitative PCR. In parallel, mice were intranasally infected with CRKP with or without rCGRP, and bacterial burden was evaluated in the lungs and extra-pulmonary sites along with assessment of inflammatory mediators in lung tissues. Our in vitro data suggests that CGRP promotes endothelial activation during CRKP infection, marked by increased expression of VCAM1, VEGF, and NLRP3. It also increased disruption of endothelial barrier integrity as judged by VE-cadherin immunostaining, and the endothelial permeability. These findings identify CGRP as a key regulator of pulmonary endothelial responses during CRKP pneumonia and suggest that neuronal signaling contributes to endothelial dysfunction during bacterial infection. Understanding CGRP-mediated regulation of endothelial function may provide new insights into host-directed therapeutic strategies for multidrug-resistant bacterial pneumonia.

39. Strain background is a key determinant of quorum sensing-dependent interspecies crosstalk in *Pseudomonas aeruginosa.* **Ahnaf Tahmid Saqif**

Quorum sensing (QS) is a population-density-dependent mechanism utilized by many bacteria to conduct intra- and interspecies communication. One type of QS involves the production and response to acyl homoserine lactone (AHL) signaling molecules,

which can vary in their side chain moiety. AHLs are produced at low levels, diffuse in and out of the cell, and increase as the population density increases. Once the AHLs reach a sufficient concentration, they bind to and activate their cognate receptor protein and cause it to activate expression of QS-regulated genes. Some AHL receptors have broad selectivity and can respond to a variety of AHL molecules, raising the possibility of AHL-dependent interspecies crosstalk. *Pseudomonas aeruginosa* is a model organism for studying QS, in part because it harbors three AHL-type QS networks: LasIR, RhlIR, and QscR. In a recent study, *P. aeruginosa* was shown to sense and respond to AHLs produced by *Burkholderia multivorans* via the RhlR receptor to increase virulence during coinfections of worms. Here, we explore how receptor selectivity varies in two *P. aeruginosa* strains. We use a GFP-based reporter to measure RhlR activation in the presence of AHL molecules with varying side chain moieties. The results show that RhlR selectivity varies dramatically between two standard lab strains, PAO1 and PA14, despite these strains having identical RhlR coding and promoter sequences. We are currently exploring the genetic and mechanistic explanation for this difference. Overall, this study offers insight into the mechanisms underlying QS-driven interspecies crosstalk, with potential relevance to polymicrobial infections.

40. A genetic screen to identify mutations that modulate the effects of quorum sensing on antibiotic resistance in the bacterial pathogen Pseudomonas aeruginosa. **Vanessa M. Schmidt**

    Pseudomonas aeruginosa is a multi-drug resistant pathogen that causes severe infections in immunocompromised hosts. Quorum sensing (QS), a cell-to-cell communication system involving a small diffusible signal and a signal-responsive transcription factor, regulates virulence and antibiotic resistance in *P. aeruginosa* and is a potential target for novel therapies. Paradoxically, QS-deficient mutants with mutations in the QS signal receptor lasR are frequently isolated from chronic *P. aeruginosa* infections in antibiotic-treated patients. Our previous work identified a mutation in the ribosome accessory factor EF-G1A that unexpectedly increased antibiotic resistance of lasR mutants, suggesting complex interactions between host adaptation and antibiotic resistance. My aim is to identify and characterize additional genetic mutations that interact with lasR to modulate antibiotic resistance in *P. aeruginosa*. A transposon mutant library will be generated in a *P. aeruginosa* signal synthase mutant (ΔlasI) and this pooled library will be screened for mutations affecting antibiotic susceptibility in a QS-dependent manner. Promising mutants will be further characterized using genomic, genetic, and biochemical approaches to elucidate the mechanisms underlying these interactions. This research will provide new insights into LasR function, *P. aeruginosa* physiology, and the evolution of antibiotic resistance in

chronic infections. Our findings may inform the development of novel anti-infective strategies targeting quorum sensing in *P. aeruginosa*.

41. PEP-R619W Differentially Modulates IFN-I Signaling to Enhance Antiviral Immunity. **Anam Shaikh**

Introduction/Rationale:
Persistent Type I interferon (IFN-I) signaling sustains chronic viral infection by inducing expression of negative regulators that suppress immune activation and drive T cell exhaustion. The autoimmune-associated variant PEP-R619W changes immune signaling, yet its impact on the IFN-I-driven pathway remains unclear. We hypothesized that PEP-R619W differentially regulates IFN-I signaling, thereby modulating antiviral immunity.

Methods:
Using CRISPR/Cas9-engineered PEP-R619W C57BL/6 mice, we analyzed protein and cytokine expression by flow cytometry, IFN-signaling via phospho-flow (pSTAT1/pSTAT4), and interferon-stimulated genes (ISGs) by real-time PCR following IFN-β stimulation.

Results:
PEP-R619W Dendritic cells (DCs) exhibited increased pSTAT1 and ISG expression with reduced SOCS3, indicating sustained IFN-I signaling and decreased negative regulation. Conversely, PEP-R619W effector CD8 T cells showed reduced pSTAT1/pSTAT4 expression, but maintained ISRE and GAS driven ISG induction, despite increased Usp18. This suggests preferential attenuation of chronic IFN-I signaling with preserved transcriptional responsiveness. Functionally, these cells produced higher levels of IFN-γ and Granzyme B, particularly in response to the minor gp276-284 epitope of LCMV-cl13, reflecting enhanced effector function and reduced exhaustion.

Conclusion:
PEP-R619W modulates IFN-I signaling in a cell-type-specific manner. It enhances DC activation while preserving the function of CD8 T cells as effector cells. This prevents immune dysfunction and enhances viral clearance. Our data highlight a novel mechanism by which the autoimmune-associated allele, PEP-R619W, rewires sustained IFN-I signaling in a cell-type-specific manner, uncovering potential therapeutic avenues for chronic virus infection.

42. APC loss enhances proteasome dependent repair of Topoisomerase II poison–induced DNA damage. **Azeem Talabi**

    Metastatic colorectal cancer (CRC) has a 5-year survival rate of ~14%, and chemotherapy offers limited benefit. Nearly 80% of CRC cases harbor mutations in the tumor suppressor Adenomatous Polyposis Coli (APC), best known as a regulator of Wnt signaling but increasingly implicated in genomic instability and DNA damage responses (DDR). DNA topoisomerase II (TopoII), which resolves replication- and transcription-associated topological stress, forms transient TopoII–DNA cleavage complexes (TOPcc). TopoII poisons, a mainstay of cancer chemotherapy, stabilize these complexes, transforming an essential enzymatic process into a source of persistent double-strand breaks (DSBs). Despite their efficacy in other malignancies, TopoII poisons have shown limited clinical benefit in CRC. Using pharmacogenomic analysis and in vitro assays, we identify APC as a key determinant of TopoII poison sensitivity. We demonstrate that APC interacts with TopoIIα and inhibits proteasome-dependent processing of TOPcc, thereby sustaining cytotoxic DSBs; APC loss accelerates TOPcc clearance and enhances damage sensing and repair independent of canonical Wnt signaling. Notably, proteasome inhibition increases TopoII poison sensitivity in APC-mutant cells. Together, these findings suggest a mechanism by which APC loss drives TopoII poison resistance in CRC and a rationale for proteasome inhibition as a therapeutic combination strategy.

43. The Microbiota-Derived Metabolite 3,4-Dimethylbenzoic Acid Inhibits *Salmonella enterica* Virulence Independently of Two-Component Systems. **Chukwuma G. Udensi**

    Human guts harbor a vast community of microorganisms known as the gut microbiome, which plays a crucial role in human health by preventing pathogen invasion. Gut commensals achieve this by competing for nutrients, attachment sites, and other known and unknown mechanisms. Previously, small-molecule extracts from human feces have been shown to modulate the expression of multiple virulence genes in Salmonella enterica. One such bioactive molecule, 3,4-dimethylbenzoic acid (DMB), was identified from fecal extracts using High-Performance Liquid Chromatography (HPLC) and mass spectrometry. Here, we confirm that DMB suppresses virulence gene expression; in particular, DMB represses the expression of genes encoded within Salmonella Pathogenicity Islands 1 and 2 (SPI-1 and SPI-2), including the master virulence regulator HilA, which is important for the ability of *Salmonella* to invade host cells. Consistent with reduced virulence gene expression, DMB-exposed *Salmonella* demonstrated 2.60-fold less invasion of HT-29 epithelial cells and 2.0-fold less invasion of RAW264.7 macrophages compared to untreated cultures. However,

at 24 hours of exposure, DMB did not affect *Salmonella* intracellular survival in RAW264.7 macrophages, suggesting that DMB primarily affects *Salmonella* invasion. We also observed that DMB had no significant effect on *Salmonella* biofilm formation compared with the untreated control. Furthermore, DMB enhanced *Salmonella* motility, suggesting that the inhibition of invasion is not a side effect of reduced bacterial motility or altered biofilm development. Similarly, minimum inhibitory concentration (MIC) assays demonstrated that DMB did not alter the antimicrobial activity of polymyxin B, as *Salmonella* cultures treated with DMB exhibited MIC values comparable to those of untreated cultures. Lastly, we screened the effect of DMB on single mutants of all two-component systems of *Salmonella*, which revealed that DMB-mediated repression of hilA occurred independently of PhoPQ, SsrA-SsrB, and EnvZ-OmpR, suggesting a novel regulatory pathway. Collectively, these findings establish DMB as a microbiota-derived anti-virulence compound that suppresses *Salmonella* pathogenesis, with implications for microbiome-inspired therapeutic strategies against enteric infections.

44. Disrupting ER quality control alleviates glycosylation stress in a *Drosophila* model of the rare disease, DPAGT1-CDG. **Mandi M.L. Wild**

    Glycosylation is an essential biological pathway that involves the co- and post-translational modification of proteins, determining their structure, functionality, and folding. Mutations in glycosylation-related genes cause improper protein folding, leading to rare conditions known as Congenital Disorders of Glycosylation (CDGs). DPAGT1-CDG is an autosomal recessive disorder caused by mutations in the DPAGT1 gene, which encodes the initial enzyme in N-glycosylation. This induces protein misfolding and accumulation within the ER, thereby eliciting cellular stress responses. Patients with DPAGT1-CDG experience severe symptoms, including neurodevelopmental delays, gastrointestinal complications, and seizures. Given the high mortality rate and the absence of curative treatments, there is a pressing medical need to identify effective therapeutic options. Our laboratory utilizes *Drosophila melanogaster* and RPE-1 human cell culture to develop disease models of DPAGT1-CDG. Previously, we identified key pathways that, when disrupted, enhance *D. melanogaster* survival following DPAGT1 inhibition, notably the two major degradative routes for misfolded proteins: endoplasmic reticulum-associated degradation (ERAD) and autophagy. Our objective is to elucidate how ERAD and autophagy modulate substrate recognition under glycosylation stress and to identify a therapeutic target for a drug. We find that RNAi knockdown of ERAD components (HRD1, SEL1L, EDEM1/2, VCP) rescues the disease phenotype in vivo, suggesting that ERAD loss is beneficial under glycosylation stress in the DPAGT1 model. We hypothesize that under glycosylation stress, ERAD and autophagy are overburdened and unable to process

proteins as quickly as they are synthesized. Notably, VCP, a component of ERAD, showed improvement when knocked down and overexpressed in the disease model. VCP participates in ERAD in the ubiquitin-proteasome system and in autophagy. Furthermore, knockdown of the autophagy regulator BECLIN-1, along with ubiquitin-proteasome cofactors NPLOC4, YOD1, and PLAA, rescued the disease phenotype. Our preliminary data suggest that loss of autophagy is also beneficial under glycosylation stress in the DPAGT1 model. This research will provide insights into the interplay between glycosylation, ERAD, and autophagy, thereby enhancing our understanding of the underlying mechanisms and guiding the development of novel therapeutic strategies.

45. ptpn-22 Mutations Affect Neuronal Migration and Axon Guidance in *Caenorhabditis elegans*. **Mark Yorio**

Protein Tyrosine Phosphatase Non-receptor type 22 (PTPN22) is characterized as a key negative regulator of T-cell activation. While studying Ptpn22, researchers have Identified mutations in the gene that have been associated with human autoimmunity and anti-viral immunity in mouse models. In vertebrates, Ptpn22 is only expressed in T cells in adaptive immunity. In *C. elegans*, ptpn-22 encodes a molecule similar to vertebrate PTPN22 but is lacking the domain involved in autoimmunity and anti-viral activity. *C. elegans* ptpn-22 is expressed broadly, including in neurons and neuroblasts. This suggests that *C. elegans* PTPN-22 might represent an ancestral PTPN family function. In *C. elegans*, PTPN-22 had been shown to be involved in formation of epidermal desmosomal adhesion complexes and cuticular molting. Work here shows that PTPN-22 is required for neuronal migration and axon guidance. Null and kinase-dead mutants show significant defects in AQR and PQR neurons that result in the cell's failure to fully migrate to its stereotypic destination. ptpn-22 mutants also show defects left-right axon guidance of the VD/DD GABAergic motoneurons. Vertebrate PTPN22 is involved in T cell migration and in the formation of the "immune synapse" adhesion complex, suggesting that an ancestral role of the PTPN22 family is cell and axon migration and cell adhesion.

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