
The Eighth Annual
Postdoc Research Day
UCONN HEALTH
The Jackson Laboratory
September 19th 2024

Welcome to the eighth annual UConn Health/Jackson Laboratory Postdoc Research Day. This is such an exciting day every year where we get to come together as a community to celebrate the important, exciting work that our postdocs are doing at UConn Health and the Jackson Laboratory for Genomic Medicine. Thank you to everyone for coming out to support and acknowledge these postdocs. Today, we will showcase some of the important work they are performing through a series of short Speak4Science talks and a full poster session. We also have an exciting keynote address from Dr. Brian Coombes from McMaster University. On the following pages, you will find a schedule as well as abstracts for all our presenters.

I would like to thank the Health Center Research Advisory Council for their support of this year's event as well as Stephanie Holden and Jane Tran Sills for their help in putting this all together. We are also pleased to have support from the Jackson Laboratory for Genomic Medicine and thank Sarah Wojiski, Dawn Traficante and Rowena Grainger from Jax for all their help. Finally, I would like to thank our PDRD Planning Committee:

Sama Abdulmalik (UConn Health)
Zeynep Altunay (UConn Health)
Chrysoula Argyrou (UConn Health)
Alexander Calderon (The Jackson Laboratory)
Anvar Sariev (UConn Health)
Patience Shumba (UConn Health)
Ying Tang (UConn Health)

Thank you for joining us in this celebration of our postdoctoral fellows during National Postdoc Appreciation Week.

Be well,

Christopher D. Heinen, Ph.D.
Director of Postdoctoral Affairs
UConn Health

Cover design and page logo credit: *Chrysoula Argyrou*

The Eighth

Postdoc Research Day

Thursday, September 19th, 2024

1:00	**Opening Remarks**	**Academic Rotunda**
1:05	**Speak4Science Part I**	**Academic Rotunda**
1:50	**Coffee Break**	**Rotunda Lobby**
2:20	**Speak4Science Part II**	**Academic Rotunda**
3:15	**Keynote Address:**	**Academic Rotunda**

Brian Coombes, Ph.D., F.A.A.M.

Professor and Chair

Department of Biochemistry and Biomedical Sciences

McMaster University

Navigating the Microbial Frontier: My path to unraveling host-pathogen dynamics in the gut

4:30	**Poster Session and Reception**	**Rotunda Lobby**
4:40	**Poster Presentations I (Odd numbers)**	**Rotunda Lobby**
5:20	**Poster Presentations II (Even numbers)**	**Rotunda Lobby**
6:00	**End**	

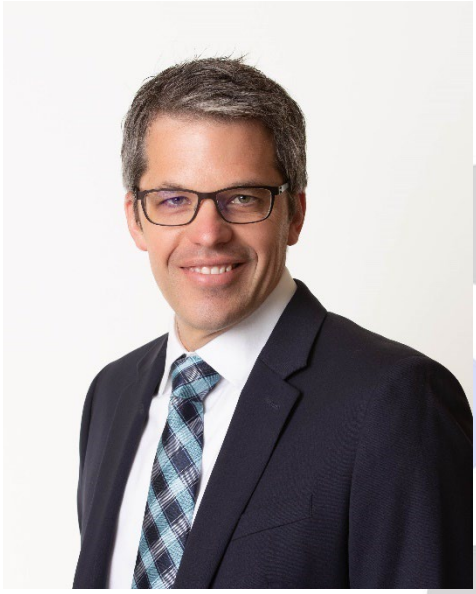
Our Speak4Science event will feature a series of 4-minute talks by our postdocs. Each speaker will use one slide to broadly introduce their area of research and why it excites them. To learn more about the details of their research, I encourage you to visit the poster sessions starting at 4:30 PM. The speaker roster and their corresponding poster numbers are listed below.

Name	Title	Affiliation	Poster
1. Anvar Sariev	*Accessing memory in mice via VR-goggles*	UCH	18
2. Moriah Turcotte	*ß-adrenergic receptors in heart disease*	UCH	1
3. Sanjeev Yadav	*Anti-miRs for ischemic stroke therapy*	UCH	2
4. Lisa Wren	*Base editing in dilated cardiomyopathy*	JAX	3
5. Aditya Kanwal	*The Titan of dilated cardiomyopathy*	JAX	4
6. Patience Shumba	*Inflammasome regulation by* Salmonella	UCH	7
7. Katherine Arnold	*Effect of ethanol on collagen I scaffolds*	UCH	9
8. Sama Abdulmalik	*Scar reduction in soft-tissue wound healing*	UCH	10
9. Manaswee Barua	*PPHOS-PLGA blends for bone regeneration*	UCH	12
10. Ying Tang	*CLR deletion regulates bone fracture healing*	UCH	13
11. Ashok Cheemala	*TDP-43 mutation causes BBB dysfunction*	UCH	14
12. Ayano Hatori	*Lacunar-canalicular network in CMD*	UCH	15
13. Suranji Wijekoon	*Therapeutic potential of small molecules on nerve injury*	UCH	16
14. Anirudhya Lahiri	*Psychosine alters astrocyte secretome*	UCH	17
15. Alexander Calderon	*3'UTR in AML differentiation*	JAX	20
16. Xuan Ye	*Evolution of Ptbp1 binding across genomes*	UCH	21
17. Amit Gupta	*Cis-elements and translation regulation*	UCH	22
18. Nisha Mahey	*RecA aids survival in* S. aureus *persisters*	UCH	24
19. Rafael Ricci de Azevedo	*Mechanism of Shiga toxin-mediated cell death*	UCH	25

Keynote Presentation

Brian Coombes, Ph.D. F.A.A.M.

Navigating the Microbial Frontier: My path to unraveling host-pathogen dynamics in the gut



Brian Coombes is Professor and Chair in the Department of Biochemistry and Biomedical Sciences at McMaster University. His research interests are centered on infectious diseases and public health, with a focus on gut health and the roles of microbes in chronic gut disorders. His lab has a strong interest in understanding bacterial virulence factors and their action on the host innate immune system. When possible, they take a 'mouse to molecules' approach to understand host-microbe interactions at a pre-clinical level, down to a careful dissection of the molecular events that dictate infection outcome. This approach has uncovered essential mechanisms of bacterial pathogenesis and led to the development of tractable systems to examine the underlying biology using genomics, proteomics and immunological methods. In addition to federal and provincial funding support that has totaled over $46M, his group has active collaborations with the private sector on novel antibiotic drug discovery and immunomodulatory approaches to treat Crohn's disease.

Dr. Coombes is the recipient of the Scientific Merit Award from the Public Health Agency of Canada, the Fisher Scientific Award for Career Achievement, and the Boehringer Ingelheim Investigator Award in Biological Sciences and was inducted into Canada's Top 40 Under 40. In 2023, he was named a Fellow of the American Academy of Microbiology. His research has been supported by industry, charitable foundations, and the public sector totaling over $46M. Dr. Coombes has published over 220 peer-reviewed articles and conference papers and given 80 invited talks worldwide on his research related to IBD and host-pathogen interactions. The Coombes lab has trained 33 graduate students, 10 postdoctoral fellows, and over 60 undergraduate students.

Abstracts

1. Moriah Turcotte, Ph.D.

Perinuclear β-adrenergic receptors are necessary and sufficient to promote cardiac hypertrophy

Moriah Turcotte Ph.D.[1], Anne-Maj Samuelsson Ph.D.[2], Sofia M. Possidento M.S.[1], Jinliang Li Ph.D.[2], Zhuyun Qin Ph.D.[2], Michael Kapiloff M.D., Ph.D.[2], Kimberly Dodge-Kafka Ph.D.[1]

[1] Calhoun Center for Cardiology, Department of Cell Biology, UCONN Health
[2] Department of Ophthalmology and Medicine, Stanford Cardiovascular Institute, Stanford University, Stanford, CA

Pathological cardiac hypertrophy development is controlled by networks of signaling pathways, integrated by scaffold proteins that localize signaling enzymes and facilitate crosstalk between these pathways, leading to upregulation of hypertrophic transcription factors. Over the last several decades, our lab has studied a protein scaffold at the nuclear envelope, muscle A-Kinase Anchoring Protein (mAKAPβ), required for the induction of cardiac hypertrophy. Among the various pathways affected, mAKAPβ particularly tethers downstream targets of β-adrenergic receptors (βAR), and this localization provides the framework for stress-related gene transcription in cardiomyocytes. Although the traditional dogma of adrenergic receptor signaling posits receptor activation begins at the plasma membrane, recent evidence has emerged showing intracellular adrenergic receptor localization and activation. We now provide evidence of a perinuclear cAMP domain that is less than 25nm in size, dependent on mAKAP expression and localization of PDE4D3 and th RII subunits of PKA. Utilizing novel peptides localized to mAKAP, we stimulated or inhibited perinuclear βARs within proximity. We demonstrate perinuclear βARs, likely expressed on the Golgi apparatus, are necessary and sufficient for cardiac hypertrophy development in neonatal and adult rat cardiac myocytes, constituting a functionally independent cAMP domain. This research has high therapeutic value as our peptides prevent disease without impacting canonical cardiomyocyte function.

2. Sanjeev Kumar Yadav

Pharmacological Validation of Advanced Gamma Peptide Nucleic Acid (PNA) Based miR Inhibitors for Ischemic Stroke Treatment

Sanjeev Kumar Yadav, PhD[1] Daylin Gamiotea Turro, PhD[1]; Vraj Patel[1]; Karishma Dhuri, PhD[2]; Raman Bahal, PhD[2] and Rajkumar Verma PhD[1]

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[2]School of Pharmacy, UConn Storrs

Our prior work demonstrated the potential of targeting miR-141-3p to mitigate ischemic stroke damage. In this study, we aimed to synthesize and validate more advanced and efficacious serine gamma peptide nucleic acid (sγPNA) based anti-miR 141-3p inhibitors, comparing them with regular PNA and phosphothiorate (PS) based counterparts. These inhibitors were subsequently encapsulated in poly(lactide-co-glycolide) (PLGA)-based nanoparticles (NPs) for treatment in a mouse model of ischemic stroke. In-house synthesis yielded PS, PNA, and sγPNA anti-miR-141-3p, encapsulated in PLGA NPs via double emulsion solvent evaporation. After physiochemical characterization, in vitro safety and efficacy were evaluated in HEK293 cells via MTT, LDH cytotoxicity assays, and qPCR for gene expression. In vivo efficacy studies employed sγPNA based anti-miR 141-3p, administered intraperitoneally 4 hours after 60-minute of transient middle cerebral artery occlusion (MCAO). Mice were sacrificed at 3 (acute) or 30 (chronic) days post-stroke. Acute cohort brain tissues were

analysed for miRNA and target mRNA levels, along with infarct volume. Chronic cohort mice were subjected to weekly behavioral task to major sensorimotor deficit or recovery. twenty-four-hour exposed HEK-293 cells were no sign of mortality and toxicity at concentration range of 0.015-1.5ug/mL of various anti-miR 141-3p. sγPNA based anti-miR exhibited notably higher efficacy in inhibiting miR-141-3p compared to PS or regular PNA. A single dose of sγPNA -based anti-miR significantly reduced infarct injury and brain tissue miR-141-3p levels (>3-fold vs. scramble control). sγPNA treatment also led to swift improvement in sensorimotor deficits in the rotarod task. We also identified TGFβ-SMAD2/3 signalling pathway as potential regulator of gamma-PNA based neuroprotection and neurorehabilitation. NPs of sγPNA-based anti-miR 141-3p were both safe and potent in vitro and in vivo. In summary, the present findings suggest that sγPNA-141 could be a potentially novel and effective therapeutic modality for the treatment of ischemic stroke.

3. Lisa Wren

Base editing correction in a mouse model of dilated cardiomyopathy

Lisa M. Wren, PhD[1], Zijian Li, BS[1], and J. Travis Hinson, MD[1,2]

[1]The Jackson Laboratory for Genomic Medicine
[2]Calhoun Cardiology Center, UConn Health

Dilated cardiomyopathy (DCM) is a disease of the heart muscle that is associated with high rates of morbidity, mortality, and healthcare costs worldwide. DCM can have a genetic pathogenesis that often progresses to heart failure and sudden cardiac death with a mortality rate approaching 50% at 5 years despite current therapies. Therefore, there is a critical unmet need to develop novel therapies to treat DCM and prevent heart failure and sudden cardiac death, particularly those that can precisely correct the underlying pathogenic gene variant identified in DCM individuals. Adenine base editors (ABE) and more efficacious generations (ABEmax) have recently been developed to initiate single nucleotide changes in targeted genes with the potential to correct pathogenic variants in inherited forms of cardiovascular disease. Therefore, we hypothesize that ABEmax delivery will repair a DCM-causing pathogenic variant in cardiac troponin T (*Tnnt2*) and improve cardiac structure and function in DCM. To test this, we used an AAV delivery system to transduce ABEmax components into cardiomyocytes to assess editing efficiency and efficacy in a mouse model of DCM. Our results show that we are able to achieve nearly 80% correction of *Tnnt2* with minimal off-target affects. We also show that ABEmax delivery rescues the DCM phenotype in our mouse model by improving cardiac structure and function. Our findings demonstrate the potential of base editors as precise gene variant correcting therapies for DCM and other inherited cardiovascular disorders.

4. Aditya Kanwal

Understanding the basis and developing a therapeutic for truncated Titin-associated dilated cardiomyopathy

Aditya Kanwal[1], Yu-Chieh Chen[2], Zijian Li[2], Nicholas Legere[2], Lisa Wren[1] and J. Travis Hinson[1,2]

[1] The Jackson Laboratory for Genomic Medicine
[2] Cardiology Center, University of Connecticut Health Center

Titin truncation variants (TTNtvs) are the most common genetic lesion identified in individuals with dilated cardiomyopathy (DCM). They are associated with high morbidity and mortality in patients. Expression of TTNtvs is accompanied by a reduction in normal Titin (TTN) protein levels, and impaired sarcomere content and function. Two models have been proposed to explain the underlying basis: Haploinsufficiency and the poison peptide. Whether one of them is responsible or there's contribution of both, remains to be conclusively addressed. Furthermore, no therapeutics currently exist that target TTNtvs due to the immense size of TTN and an incomplete knowledge of TTNtv pathogenicity. In this study, we are harnessing CRISPR technology

to target TTNtvs arising from the frameshift mutations in the Ttn gene to assess the therapeutic potential of this strategy.

5. Daylin Gamiotea Turro

Phagocytic potentials of purinergic receptor P2x4 blockade after ischemic stroke

Daylin Gamiotea Turro[1], Sanjeev K. Yadav[1], Arun K. Yadawa[1], Mary-Katherine Cormier [1], Chunxia C. Cronin[2], Bruce T Liang[2], Rajkumar Verma[1]

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[2]Calhoun Cardiology Center School of medicine, UConn Health

Ischemic stroke (IS) is a major cause of disability and the fifth leading cause of death in the United States, highlighting the urgent need for new and effective treatments beyond the acute phase. Previous studies have shown that both genetic removal and short-term pharmacological inhibition of the P2X4 receptor (P2X4R)—a purinergic receptor involved in adenosine triphosphate (ATP) signaling—provide neuroprotection by reducing acute inflammatory responses, making it a promising target for IS therapy. However, the mechanisms behind the long-term benefits of P2X4R inhibition are not well understood. In this study, we hypothesize that blocking P2X4R enhances the phagocytic clearance of necrotic or apoptotic tissue, thus accelerating recovery. To test this, we used 1µm fluorescent phagocytic beads and pHrodo Red zymosan A Bioparticles conjugate to measure in vitro and ex vivo phagocytic uptake by lipopolysaccharide (*LPS*) (200 µg/ml for 2 hours) primed Bone Marrow Derived Macrophages (BMDMs) from P2X4R knockout (KO) and wildtype (WT) mice. We also analyzed phagocytic uptake in flow sorted Ly6Chi and Ly6Clo monocytes from the perilesional ipsilateral brain tissue of P2X4R KO and WT mice at 3- and 7-days post-stroke, induced by 60 minutes of transient middle cerebral artery occlusion (MCAo). Our results show a significant increase in phagocytic uptake by BMDMs from P2X4R KO mice compared to WT mice, with a 1.5- and 2-fold increase respectively ($P < 0.05$, Student's t-test; n = 4 mice/group × 2 technical replicates). Additionally, infiltrating Ly6Chi inflammatory monocytes from P2X4R KO mice had significantly higher phagocytic bead uptake than WT controls (*$P < 0.05$). These monocytes also displayed increased expression of CD36, a scavenger receptor marker, at 7 days post-stroke, suggesting an enhanced phagocytic phenotype. In summary, our study demonstrates that P2X4R inhibition provides rapid and sustained neuroprotection by promoting the phagocytic clearance of damaged tissue following ischemic stroke.

6. Jessica N. Flori

A preliminary investigation of alcohol expectancies in a sample of soup kitchen attendees

Jessica N. Flori,[1] Kristyn Zajac,[1] Carla J. Rash[1]

[1] *Calhoun Cardiology Center Behavioral, Behavioral Health, UConn Health*

Soup kitchen attendees are disproportionally affected by alcohol-related consequences and mortality. Although some studies have evaluated the unique intervention needs of soup kitchen attendees, cognitive variables related to alcohol use (e.g., alcohol expectancies) are understudied. This study aims to develop a preliminary understanding of alcohol expectancies among soup kitchen attendees to inform intervention targets. Participants (n = 22) were soup kitchen attendees who reported risky drinking, who primarily identified as male (n =15), African American (n = 10) or White (n = 9), and non-Hispanic (n = 20). Many were unhoused (n = 13). Bivariate statistics examined relationships between alcohol expectancy subscales and alcohol use and related consequences. Results revealed sexuality, (e.g., I would enjoy sex more; r = .46, p = .030) and self-perception (e.g., I would feel self-critical; r = .64, p = .002) expectancies were positively correlated with alcohol problem severity (M = 15.86). Cognitive behavioral impairment (e.g., My senses would be dulled; r =

.519, p = .013) and self-perception (r = .68, p = .001) expectancies were also positively correlated with alcohol-related consequences (M = 14.41). Results suggest that alcohol expectancies of soup kitchen attendees are unique with not all subscales being correlated with alcohol use and problems. Cognitive interventions that target positive expectancies may be less helpful in reducing alcohol use for this population. Results contrast extant expectancy literature that suggests positive expectancies are most closely related to risky drinking in general population adults and college students.

7. Patience Shumba

Activation of the non-canonical inflammasome by a *Salmonella* type three secretion system effector

Patience Shumba[1], Sivapriya Kailasan Vanaja[1], Vijay Rathinam[1]

[1] Department of Immunology, UConn Health

Salmonella type three secretion system (T3SS) effectors are important virulence factors promoting both invasion of host cells and intracellular bacterial replication. Intracellular *Salmonella* is mostly found within *Salmonella* containing vacuoles (SCV), however the bacteria can also escape into the cytosol. In the cytosol, lipopolysaccharide (LPS) on bacterial membranes is sensed by the non-canonical inflammasome activating pyroptosis, an inflammatory type of cell death. Non-canonical inflammasome activation can lead to endotoxemia and sepsis associated with poor clinical outcomes. T3SS effectors are known to maintain SCV integrity and interact with host proteins promoting pro- and anti- inflammatory responses in host cells. Here we aimed to evaluate the effect of *Salmonella* T3SS effectors on non-canonical inflammasome activation in epithelial cells. To this end, we infected epithelial cells with single gene-deletion mutants of *Salmonella* T3SS effectors and measured cell death, expression of caspase-4, the protease which senses cytosolic LPS and triggers pyroptosis. We observed a significant reduction in cell death and activation of caspase-4 in epithelial cells infected with a mutant for a T3SS effector, *Salmonella* secretion effector L (SseL) a deubiquitinase. Interestingly, SseL does not contribute to invasion of epithelial cells, intracellular bacterial replication and vacuolar escape of the bacteria. Our results suggest that SseL plays an important role in non-canonical inflammasome activation. We are currently addressing the mechanism by which SseL contributes to non-canonical inflammasome activation.

8. Yusuke Kondo

Outer arm dynein intermediate chain 2 regulates ciliary motor activity

Yusuke Kondo[1], Maya Yankova[2], Stephen M. King[1 2]

[1]Department of Molecular Biology and Biophysics, UConn Health
[2]Electron Microscopy Facility, UConn Health

Cilia are hair-like motility organelles found in eukaryotic cells. Cilia move like a whip, allowing cells to swim and transport materials. Cilia dysfunction can cause infertility and chronic bronchitis, so research into ciliary motility is also important medically. Cilia are complexes of over 600 proteins, and their basic motility mechanism is caused by the sliding movement of microtubules and the motor protein dynein, which is densely arranged on them. Because this sliding movement propagates at high speed, it is thought that dynein has a high-speed switching mechanism, but the details are unknown. To clarify this high-speed switching mechanism, we conducted research using the green alga Chlamydomonas reinhardtii, a model organism for cilia research. First, we hypothesized that the outer arm dynein intermediate chain 2 (IC2) is important for the high-speed switching of dynein. This is because recent structural studies have shown that IC2 contacts the dynein motor domain and is detached during the ATP hydrolysis cycle. Protein structural information has revealed that IC2 electrostatically interacts with the motor domain. We therefore investigated how the electrostatic interaction between IC2 and the motor domain contributes to ciliary motility by creating mutants

K355E and D352K, which weaken and strengthen the electrostatic interaction by introducing point mutations into IC2. The swimming velocity of K355E was reduced to 30% of that of the wild type, while that of D352K was comparable to that of the wild type. Furthermore, in a highly viscous environment, D352K exerted a higher propulsive force than the wild type. These results indicate that the electrostatic interaction between IC2 and the motor domain has a strong effect on the motor activity of dynein. In the future, we plan to investigate whether this change in motor activity is due to a high-speed switching mechanism.

9. Katherine Arnold

Characterization of ethanol post-treatment on collagen type I scaffolds for pathological tendon modelling

Arnold, KM[1]; Deymier, AC[1]

[1]Biomedical Engineering, School of Dental Medicine, UConn Health

Tendons are comprised almost entirely of type I collagen, making scaffolds an ideal model for *in vitro* studies. To develop a model of tendon pathology, we are characterizing the behavior of collagen I scaffolds with ethanol (etOH) post-treatment and hydration. Ethanol dehydration is an extremely common step in both scaffold manufacture and tissue processing, and yet the influence of etOH on collagen is not well understood. Collagen I scaffolds were submerged in single etOH dilutions, serial dilutions, or deionized water overnight, at which point they were air dried completely to remove remaining solvent. At this stage Raman spectroscopy showed no significant changes to the structure of the collagen triple helix, but Fourier Transform Infrared spectroscopy (FTIR) indicated that etOH treatment altered the secondary structure, leading to increased fibrillogenesis. This was indicated by a downshift of the Amide II and III peaks (1540 and 1236cm^{-1} respectively), and a broadening of the Amide I peak at 1640cm^{-1}. All samples had a very broad series of peaks between 2500 and 3750cm^{-1} indicative of tightly bound water. The area of these peaks decreased with exposure to etOH. SEM revealed no differences in the macroscopic structure between water and etOH treated samples, with all scaffolds showing an extremely dense fibrillar structure, similar to that of tendon. These results indicate that the effect of ethanol is limited to the secondary structure of collagen, rather than the molecular or macroscopic levels. Thermogravimetric analysis (TGA) showed that ethanol treatment decreased the thermal stability of collagen scaffolds, causing them to break down at lower temperatures, and causing increased mass loss between key inflection points. The most profound differences caused by etOH exposure were found in the rehydration properties. Ethanol treatment, even up to two weeks previously, results in significantly increased swelling upon rehydration in deionized water and eventual loss of scaffold structure with hyper-hydration. The current hypothesis is that ethanol causes “clumping” of collagen fibers, increasing inter-fibrillar spacing and therefore rehydration capacity. This study is essential to understanding the interaction of collagen and ethanol, both from a basic science perspective and to improve scaffold manufacture.

10. Sama Abdulmalik

Electrical and Chemical Stimulation Using Ionically Conductive Polymeric Implants for Soft Tissue and Wound Healing

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[1]Department of Orthopedic Surgery, UConn Health
[2]Department of Biomedical Engineering, University of Connecticut, Storrs, CT

Musculoskeletal injuries require surgical intervention, forming scar tissue during the natural healing. Scar tissue, characterized by a disorganized fibrotic extracellular matrix with altered components, often hinders tissue regeneration. To address this challenge, we have developed an ionically conducting (IC) chitosan

scaffold that serves as a matrix for delivering electrical stimulation (ES) and pharmacological agents to promote regenerative healing while minimizing scar formation. Our IC implant promotes the benefits of ES with the controlled release of a small molecule, 4-aminopyridine (4AP), to enhance regenerative healing and reduce scarring. Our IC matrix maintained excellent ionic conductivity (130-145 mS/cm for 12 weeks) and sustained the release of the highly water-soluble 4-AP over eight weeks. In an experimental model using rats with full-thickness skin wounds, we found that wounds treated with ES+4-AP implants exhibited significantly faster closure rates and a higher Col-I/Col-III ratio, indicating enhanced regeneration and reduced scar formation. We further assessed the progress of wound healing by evaluating the neurotrophic expression and cytokeratin proteins. These findings hold promise in addressing the growing issue of musculoskeletal injuries and improving the overall well-being of affected individuals, providing a reassuring and confident outlook for the future.

11. Chinedu Ude

Novel Chelation Therapy for the Treatment of Arthroplasty-related Metallosis

Chinedu C. Ude [1,2] *Taraje Whitfield*[1,2], *Cato T. Laurencin* [1,2,3,4,5,6,7*]

[1]*The Cato. T Laurencin Institute for Regenerative Engineering,* [2]*Department of Orthopaedic Surgery, University of Connecticut Health,* [3]*Department of Chemical and Biomolecular Engineering, University of Connecticut, Storrs, CT,* [4]*Department of Biomedical Engineering, University of Connecticut, Storrs, CT,* [5]*Department of Materials Science and Engineering, University of Connecticut, Storrs, CT,* [6]*Institute of Materials Science, University of Connecticut, Storrs, CT,* [7]*Department of Craniofacial Sciences, School of Dental Medicine, University of Connecticut Health*

Introduction: Cobalt-containing alloys are useful for orthopedic applications due to their low volumetric wear rates, corrosion resistance, high mechanical strength, hardness, and fatigue resistance. Unfortunately, discoveries after their widespread implantation indicated that these prosthetics release significant levels of cobalt ions, into patients requiring replacements.
Methods: A synovial fluid-mimicking chelator was designed. Hyaluronic acid (HA), the major chemical component of synovial fluid, was functionalized with British anti-Lewisite (BAL) to create a novel chelator (BAL-HA). Furthermore, A novel metallosis animal model was created and treated by injecting cobalt II ions into the hip joint to allow for tissue toxicity similar to the hip toxicity of a malfunctioning metal implant.
Results: BAL-HA effectively binds cobalt and rescues in vitro cell vitality (up to 370% of cells exposed to IC_{50} levels of cobalt). In vivo data depicted that BAL-HA enhances the rate of cobalt clearance ($t_{1/2}$) from 48 h to 6 h. Gross evaluation of hip joints revealed that cobalt group had inflammation and tissue discoloration, while BAL-HA group had well-preserved features. Quantified tissue/joint deterioration data depicted that the tissue damage in cobalt group was significantly higher compared to BAL-HA-treated group. The percentage inflammation and deterioration evaluated by H&E and IHC that occurred in cobalt group were significantly higher compared to the BAL-HA. Furthermore, the ICP-MS, and small molecule off-target effect evaluations for the internal organs (spleen, heart, kidney, and liver) indicated no significant cobalt ions in both groups, suggesting no off-target effects of BAL-HA treatment.
Discussion: A biocompatible chelator blend BAL-HA, and a novel animal model of metallosis for arthroplasty wear and corrosion debris, were successfully developed and assessed with multiple evaluations which indicated that the chelator system was efficacious in elimination of cobalt ions with no risk of complications, including kidney failure.
Conclusion: Our data indicated that BAL-HA has the potential to mitigate cobalt toxicity caused by metal ions from metallic implants with no adverse effect to the body

12. Manaswee Barua

Polyphosphazene-PLGA blends for bone regeneration

Manaswee Barua[1] and Cato T. Laurencin[1,3,4,5,6,7]

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Bone is a dynamic tissue with an intrinsic capacity to regenerate. However, if the bone defect is above a critical size, the regeneration process needs to be surgically augmented. The bone graft substitutes clinically available for reconstruction surgeries can be broadly classified into autografts, allografts, and synthetic bone graft substitutes. Polyphosphazenes are an interesting class of inorganic-organic hybrid biodegradable polymers with synthetic flexibility, metabolizable by-products, tunable physical and chemical properties, and low *in vitro* and *in vivo* cytotoxicity. A polyphosphazene-PLGA blend is stable under ambient conditions and the fabricated synthetic graft has mechanical properties comparable to the native bone. This polyphosphazene-PLGA graft has been successfully studied for the regeneration of a critical-sized defect (1.5 cm) in the radius bone of rabbits. This study was performed without the addition of any cells or external growth factors into the graft. Moreover, this polyphosphazene-PLGA blend undergoes a unique *in vivo* degradation via an *in-situ* pore formation mechanism which makes the fabrication of these blends simpler and opens the possibility of a faster bench to bedside translation.

13. Ying Tang

CLR Deletion in Endothelial Cells Regulates Bone Fracture Healing

Ying Tang[1], Sanja Novak[1], Ivo Kalajzic[1]

[1]Center for Regenerative Medicine and Skeletal Development, School of Dental Medicine, University of Connecticut Health Center

Calcitonin gene-related peptide (CGRP), a neuropeptide, plays various neuroendocrine roles such as vasodilation and pain modulation. Studies have indicated effects of CGRP on enhancing bone formation, inhibiting bone resorption, and promoting vascular growth. CLR (gene *Calcrl*) dimerizes with the receptor activity modifying protein 1 (RAMP1) to create CGRP receptor for signaling. We previously demonstrated expression of *Calcrl* in periosteal and endothelial cells (ECs). When we used CGRP inhibitor or deleted CLR in aSMA osteoprogenitors, fracture healing was impaired, but underlying cellular mechanisms are undefined. In this study, we investigated the effects of targeted CLR deletion in ECs on bone fracture healing.

We have bred Cdh5-CreER with $CLR^{fl/fl}$ mice to selectively target deletion to ECs. To induce CLR deletion, both male Cre^{-} and Cre^{+} mice were treated with tamoxifen on -2/0/2 days post fracture (DPF). We confirmed the reduced gene expression of *Calcrl* and *Ramp1* in 7DPF callus tissue of Cre^{+} mouse. We have completed single cell 10X seq of $CD45^{-}/Ter119^{-}$ cells isolated from periosteum callus at 4DPF from $Cdh5Cre/CLR^{fl/fl}$ mice (Cre^{-} & Cre^{+}). Unsupervised clustering determined undifferentiated mesenchymal (MSCs), chondrocyte, osteoblasts, endothelial cells, satellite cells, smooth muscle cells, and tenocytes clusters. Deletion of CLR within ECs led to significantly decreased expression of multiple genes such as *Col1a1, Col2a1, Ibsp, Acan, Comp* in MSCs, osteoblast and chondrocyte clusters. Gene Set Enrichment Analysis (GSEA) detected multiple down-regulated biological pathways from Gene Ontology (GO) including mesenchymal cell differentiation, ossification, cartilage development, chondrocyte differentiation, skeletal

system morphogenesis. KEGG pathway analysis showed down-regulation of ECM-receptor interaction and PI3K-Akt signaling in those clusters from Cre^+ mouse, suggesting suppressed cell proliferation activity. To verify this, histological analysis of Ki67 staining was performed at 4DPF, revealing lower number of $Ki67^+$ cells (p=0.06) in Cre^+ calluses.

Our data present that deletion of CLR in ECs suppresses mesenchymal differentiation, chondrogenesis, osteogenesis during bone fracture healing process, indicating that CLR deletion in endothelial cells impairs the activity and function of mesenchymal progenitor cells, which potentially results in impaired bone fracture healing process.

14. Ashok Cheemala

Single-allele TDP-43^{G348C} Mutation Leads to Endothelial Dysfunction and Blood-Brain Barrier Defects in Mouse Models of Amyotrophic Lateral Sclerosis and Frontotemporal Dementia.

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Loss of nuclear TDP-43 occurs in a wide range of neurodegenerative diseases, and specific mutations in the *TARDBP* gene that encodes the protein are linked to familial Frontal Temporal Lobar Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS). Although researchers have focused on neuronal cell dysfunction caused by TDP-43 variants, *TARDBP* mRNA transcripts are expressed at similar levels in brain endothelial cells (ECs). Since increased permeability across the blood-brain barrier (BBB) precedes cognitive decline, we postulated that altered functions of TDP-43 in ECs contributes to BBB dysfunction in neurodegenerative disease. To test this hypothesis, we examined EC function and BBB properties in mice with one of several mutations: knock-in *TARDBP^{G348C}*, a mutation found in ALS/FTLD patients; *Cdh5(PAC)CreERT2; Tardbp^{ff}*, in which TDP-43 is deleted throughout endothelium in the entire organism; and *Slco1c1(BAC)CreERT2; Tardbp^{ff}*, in which TDP-43 is specifically deleted within brain endothelium. We found that *TARDBP^{G348C}* mice exhibited increased permeability to 3kDa Texas Red dextran and NHS-biotin, relative to their littermate controls, results we recapitulated in cultured brain ECs taken from these mice. Nuclear levels of TDP-43 were reduced *in vitro* and *in vivo* in ECs from *TARDBP^{G348C}* mice. This coincided with a reduction in junctional proteins VE-cadherin, Claudin-5, and ZO-1 in isolated ECs, supporting a cell-autonomous effect on barrier function through a loss of nuclear TDP-43. We modeled endothelial-cell-specific loss of TDP-43 using *Cdh5(PAC)-CreERT2* and *Slco1a1(BAC)-CreERT2* deletion and found that the loss of TDP-43 throughout the endothelium led to systemic endothelial activation and permeability. Deletion specifically within the brain endothelium bypassed systemic complications, and demonstrated acutely increased BBB permeability, and eventual hallmarks of FTD, including fibrin deposition, microglial and astrocyte activation, and behavioral defects. Together, these data show that TDP-43 dysfunction specifically within brain ECs would contribute to the BBB defects observed early in the progression of ALS/FTLD.

15. Ayano Hatori

Disruptive lacunar-canalicular network in a $Cx43_{R239Q}$ knock-in mouse model for autosomal recessive craniometaphyseal dysplasia

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Craniometaphyseal dysplasia (CMD) is a rare genetic disorder characterized by progressive thickening of craniofacial bones and flared metaphyses of long bones. A missense mutation in Connexin43 ($Cx43_{R239Q}$) causes the autosomal recessive (AR) CMD. We have generated a $Cx43_{R239Q}$ knock-in (KI) mouse model (*$Cx43^{KI/KI}$*), which replicates many features of AR CMD. Cx43 is a major hemichannel and gap junction protein to facilitate communication and exchange of small molecules in bone. The lacunar-canalicular network (LCN) of bone harboring osteocytes and their dendritic outgrowths plays an important role in mechanosensing during bone modeling and remodeling. To study $Cx43_{R239Q}$ mutational effects on osteocytes, we performed single-cell RNA-seq of cells isolated from femoral cortical bones. Clusters of cells with the highest expression of *Dmp1*, *Mepe*, and *Phex* were considered osteocytes. We showed that solute carrier genes, *Slc3a2*, *Slc39a8*, and *Slc35f5*, are significantly reduced in *$Cx43^{KI/KI}$* osteocytes, suggesting that the transporting activity of small molecules, such as amino acids, zinc, and nucleotides, may be compromised. We next examined dendritic extensions and morphology of osteocytes by rhodamine-phalloidin staining and scanning electron microscopy. Our data showed that the perilacunar space of *$Cx43^{KI/KI}$* osteocytes is increased and the number and length of dendrites are remarkably decreased. We further examined mechanosensors expressed in osteocytes and dendrites. While levels of *Cx43*, *Panx1*, and *P2X* receptors are comparable between *$Cx43^{+/+}$* and *$Cx43^{KI/KI}$* littermates, P2Y purinergic receptors *P2RY1*, *P2RY12*, and *P2RY13* are significantly decreased in femoral cortical bones of *$Cx43^{KI/KI}$* mice. Consistent with the role of P2Y in inhibition of bone formation, we observed increased bone formation on the periosteum of cortical bones and focal bone nodules. Taken together, our data suggest that the $Cx43_{R239Q}$ mutation alters the LCN of cortical bone. This study provides a possible pathogenic mechanism for AR CMD by linking the disruption of the LCN to its cortical phenotype.

16. Suranji Wijekoon

Therapeutic potential of small molecule in promoting neural tissue regeneration and functional recovery following peripheral nerve injury

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Peripheral nerve injury (PNI) remains an enormous clinical challenge while drastically affecting the quality of the life of the patient. This study focuses on addressing the challenges of PNI by exploring tissue engineering solutions for nerve regeneration, using a rat sciatic nerve defect model. Traditional treatments like autografts and allografts come with several drawbacks such as limited availability, immune response issues, and complications at the donor site. The research proposes an alternative approach using small molecules to stimulate Schwann cells (SCs) through the Wnt pathway, promoting nerve repair without the limitations of biological grafts. The study's *in vitro* phase involved treating SCs with small molecules (SGK1, 2, 3, 4, 5), followed by viability assays, immunostaining, and PCR analysis to assess the Wnt pathway's activation and the cells' response. Results indicated that the small molecules effectively activated the Wnt pathway and enhanced Schwann cell activity. In the *in vivo* phase, surgical defects were created in rat sciatic nerves, and scaffolds containing small molecules were implanted to evaluate their impact on regeneration. Histological analyses demonstrated improved axonal growth, SCs infiltration, and nerve tissue regeneration in comparison to control groups. Functional assessments, including motor and sensory recovery tests, further supported the

conclusion that small molecule-loaded scaffolds significantly improved recovery outcomes. Molecular analysis reinforced the involvement of the Wnt pathway, highlighting its mechanistic role in nerve repair. In summary, the findings suggest that small molecules stimulating the Wnt pathway through SCs activation hold significant promise for developing new clinical strategies in PNI treatment. The study provides evidence that this approach could overcome the limitations of current biological grafts, improving nerve regeneration and patient outcomes.

17. Anirudhya Lahiri

Psychosine alters the neuroinflammatory astrocyte secretome

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Globoid cell leukodystrophy (GLD) also known as Krabbe's disease, is a rare fatal lysosomal storage disease. In GLD, loss of function mutations in the galactosylceramidase (GALC) gene results in pathological accumulation of a sphingolipid named psychosine in the central nervous system (CNS), which plays a causal role in driving neuropathology. Early activation of glial cells (astrocytes, microglia) and aberrant upregulation of inflammatory cytokines and chemokines in CNS are pathological hallmarks of GLD. However, the contribution of astrocytes in driving neuroinflammation and subsequent neuropathology in GLD is poorly characterized. Here, we examined how psychosine regulates astrocytic inflammatory gene expression profile using an unbiased approach. We found that, psycosine treatment alone induced expression of cytokines interferon (IFN)-γ, interleukin (IL)-6, granulocyte macrophage- colony stimulating factor (GM-CSF) and chemokine C-X-C Motif chemokine Ligand (CXCL)-1. Furthermore, astrocytes treated with other proinflammatory stimuli IL-1β or lipopolysaccharide (LPS) alone, resulted in increased production of these same factors (IFN-γ, IL-6, GM- CSF, CXCL1). IL-1β treatment alone additionally induced C-C motif chemokine ligand (CCL)-5 production, whereas LPS treatment found to elevate production of CCL3 and IL-12p40/p70. Thereafter, we investigated whether psychosine modulates astrocytic neuroinflammatory gene expression profile induced by IL-1β or LPS. We found that psychosine did not significantly alter IL-1β induced gene expression. In contrast, psychosine in combination with LPS was found to enhance astrocytic secretion of GM-CSF. Taken together, these preliminary data indicate that psychosine alone does not appear to significantly modify inflammatory gene expression in astrocytes whereas it may amplify the inflammatory activity of astrocytes in response to coincident inflammatory stimuli. Our data also indicate that the elevated presence of psychosine in GLD can modify the dynamic response of astrocytes to other inflammatory stimuli which might play an important role in modulating monocytes and or T cell responses which have been implicated in GLD pathology.

18. Anvar Sariev

Simultaneous optical and electrical access to long-term memory ensembles in mice navigating virtual environments.

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Creating cognitive maps to navigate environments is a crucial role of the hippocampal memory system. Spatial memory deficits often accompany neurological conditions like dementia. To effectively use these maps for navigation, several functions are necessary: initial encoding of environmental layout, distinguishing between similar yet distinct environments, and identifying significant cues within each map. While cognitive maps

spontaneously form during exploration, their evolution and the underlying neural circuitry remain largely mysterious. Here, we present a novel approach to study the encoding, consolidation, and retrieval of cognitive maps in which we simultaneously combine: 1) two-photon calcium imaging, 2) large-scale electrophysiological recordings, 3) 3D virtual reality exploration, 4) tracking movement and lick rate, and 4) real-time pupillometry. Complementing the calcium imaging of thousands of neurons tracked across weeks, simultaneous electrophysiological recording across the entire hippocampus provides temporally precise information about ongoing network states and inputs within neural circuits. Virtual reality environments offer unprecedented control over sensory inputs and behavioral paradigms, enabling precise manipulation of cognitive processes. Simultaneously tracking pupillary responses provides an independent measure of task engagement, arousal, and attention providing access to processes known to influence memory formation on sub-second timescales. Currently, we are combining this approach with a closed-loop network-state triggered optogenetics for targeted manipulations of the circuits of spatial memory consolidation. By leveraging the strengths of each technique, this novel combinatorial approach offers a comprehensive approach to the study of the neural mechanisms underlying memory processes, paving the way for targeted interventions in memory-related disorders and cognitive enhancement strategies.

19. Alexandra Pozhidaeva

Functional disorder in the N-terminus of Phosphoethanolamine Methyltransferase from *P. falciparum* studied using solution NMR spectroscopy and MD simulations

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Over 500000 people die from malaria every year. Recent emergence of drug-resistant malaria parasites has heightened the need for new treatments to complement the existing therapies. Protein methyltransferases (PMTs) represent a class of enzymes that utilize S-adenosyl methionine (SAM) to methylate protein substrates. PfPMT found in *Plasmodium falciparum* plays a key role in synthesizing phosphatidylcholine (PC), a major component of plasmodium membranes. A PC synthesis pathway in which PfPMT converts phosphoethanolamine to phosphocholine, is absent in mammals making the enzyme an appealing therapeutic target. Crystal structures of the enzyme bound to its ligands/inhibitors have been determined in recent years. Interestingly, PfPMT residues involved in ligand interactions are not solvent-exposed in these structures, suggesting that conformational changes are required for ligand recognition. Yet, the structure of the apo protein has never been crystalized suggesting conformational dynamics in absence of bound ligands.

In this study we characterized apo PfPMT enzyme using solution NMR methods in combination with molecular dynamics simulations. Our findings revealed distinct conformational differences between the apo and the ligand-bound states. Specifically, we found that N-terminus of the apo enzyme is unstructured and undergoes disorder-to-order transition during ligand binding. We also gained insight into the mechanism of PfPMT inhibition by a known inhibitor amodiaquine (AQ). AQ binds away from the active site of the enzyme and the mechanism of its action remained unclear from the crystal structure. Our study revealed that chemical shift perturbations of PfPMT residues upon addition of AQ propagate from the binding site to the active site via the N-terminus highlighting its role in functioning of the enzyme. This work provides a basis for future efforts to develop new potent anti-malarial compounds.

20. Alexander Calderon

3'UTR Processing Suppresses AML Differentiation

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Acute Myeloid Leukemia (AML) is an aggressive cancer whose hallmarks include an accumulation of blasts from the myeloid lineage. These cells are characterized by rapidly dividing cells who are blocked in their ability to terminally differentiate. First-line therapy continues to be aggressive chemotherapy regimens that include cytarabine and an anthracycline. However, there is an interest in the use of differentiation-inducing agents as a less toxic strategy for more frail patient populations. Unfortunately, drug resistance remains a persistent problem, with many patients going on to relapse. Recently, it has become appreciated that non-genetic mechanisms are a critical mechanism by which malignant cells block their differentiation and develop therapy resistance. However, there is still an incomplete understanding of gene regulatory pathways that induce differentiation and arrest leukemic cells. Here, we applied an immunophenotypic readout and positive selection CRISPR/Cas9 screens to identify suppressors of leukemic differentiation. These screens converged on ZBTB7A, a zinc finger transcription factor whose *loss-of-function* restricted AML maturation in the presence of differentiation-inducing agents. We found that genetic deletion of ZBTB7A and its paralog, ZBTB7B cooperatively suppresses myeloid differentiation and promotes resistance to several clinical compounds. Lastly, we identify that leukemic cells hijack multiple upstream 3'UTR processes, such as alternative polyadenylation and mRNA decay to downregulate *ZBTB7A* and *ZBTB7B* expression. Together, these data provide fundamental insight into non-genetic mechanisms that impede myeloid differentiation and mediate drug resistance.

21. Xuan Ye

Evolutionary conserved and divergent binding of Ptbp1 across genomes

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Genetics and Genome Sciences, UConn Health

RNA protein interactions play critical roles in all aspects of RNA metabolisms in all species. However, RNA protein interactions have not been examined at a large genomewide scale. To examine the evolutionary landscape of RNA protein interactions across genomes, we focus on Ptbp1, a highly conserved RBP involved in RNA splicing and neurodevelopment. We systematically characterized Ptbp1 binding events in human, mouse, pig and hamster cell lines using eCLIP approach. Ptbp1 in different species binds its cognate "CU" rich sequence motif, many of those binding sites are within the evolutionary conserved regions. However, Ptbp1's binding patterns are altered by variation in genome sequences, which can result in diminished binding or changed binding sites. Together, the data indicate that RBP homologs bind to evolutionary conserved binding regions in cellular conditions. According to our findings, Ptbp1's evolutionary conserved and divergent binding landscapes are not only caused by variations in the RNA binding protein, but also by variations in genomes.

22. Amit Gupta

NaP-TRAP: A tool to identify *cis*-elements regulating mRNA translation

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Cellular proteome is meticulously regulated by the changes in mRNA abundance and translation. The regulation of translational is primarily carried out by the interplay between *cis*-regulatory elements and *trans*-factors. *Cis*-regulatory elements embedded within mRNA sequences, such as 5'- and 3'-untranslated regions (UTRs), serve as docking sites for *trans*-factors, ultimately determining mRNA fate. Previous studies have identified critical elements and factors, such as poly(A) tail length and microRNAs, that regulate mRNA translation in early embryos. However, the lack of techniques that allow for high-throughput analysis of *cis*-regulatory elements on translation in dynamic systems has impeded our understanding of mRNA translation control. Here, we introduce Nascent Peptide-mediated Translating Ribosome Affinity Purification (NaP-TRAP), a novel approach to study translation regulation in dynamic systems. NaP-TRAP can capture the regulatory activity on translation for various mRNA elements such as the mRNA cap structure, UTR elements, codon optimality and poly(A) tail length. We demonstrate that NaP-TRAP is versatile for studying translation control in both dynamic systems, like developing zebrafish embryos, and steady-state systems, like human cell lines. Moreover, NaP-TRAP can be adapted into massively parallel reporter assays to uncover 5' UTR regulatory elements orchestrating mRNA translation in zebrafish embryos. In conclusion, NaP-TRAP is an easy-to-use, inexpensive, and versatile technique for uncovering *cis*-elements that control mRNA translation across various biological systems. Beyond enhancing our understanding of gene regulation, NaP-TRAP can guide approaches to predictably tune translational regulation through *cis*-element engineering.

23. Everton Bettin

Sequence variability of BamA and FadL orthologs reveals divergent evolutionary paths of Treponema pallidum outer membrane proteins

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The extent to which sequence and antigenic diversity of *Treponema pallidum* (*TPA*) outer membrane proteins (OMPs) complicates vaccine development and fuels the worldwide resurgence of syphilis remains unknown. Our group has identified *TPA* BamA and members of the FadL family as potential components of a syphilis vaccine 'cocktail'. As part of a global consortium to explore *TPA* strain diversity, we mapped the variability of BamA (TP0326) and five FadL orthologs (TP0548, TP0856, TP0858, TP0859 and TP0865) in 186 strains from Malawi, China and Colombia onto their predicted three-dimensional (3D) structures. Single nucleotide variants accounted for the majority of the OMP variability in our cohort, with notable higher variability within Nichols-like strains. Most mutations were in regions predicted to be extracellular, consistent with the notion that pressure exerted by the spirochete's obligate human host drives OMP diversity. We observed a spectrum

of variability among OMPs indicating unequal evolutionary pressures across this portion of the OMPeome. Phylogenetic analysis using concatenated proteoforms revealed a heterogeneity of OMP profiles. A close relationship was observed between OMP profiles within each *TPA* subclade, with distinct OMPs and/or regions within the proteins driving profile variability in each lineage. This findings suggest that demographic factors influence the evolution of *TPA* OMPs. Our study provides new insights into the forces shaping the host-pathogen interface of the syphilis spirochete on a global scale and informs the selection of ECL targets for vaccine design.

24. Nisha Mahey

Timing matters: *recA* facilitates DNA damage repair in *S. aureus* persisters following fluoroquinolone treatment

Nisha Mahey[1], *Jonathan I. Batchelder*[1], *Wendy W. K. Mok*[1]

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Bacterial persisters represent distinct phenotypic variants within genetically identical populations, which are capable of surviving concentrations of antibiotics that kill their kin. Given their potential to survive antibiotic treatment, persisters are important contributors to infection recurrence and resistance development, including infections involving *Staphylococcus aureus*. Antibiotic tolerance in persister cells is often associated with reduced activity of antibiotic targets, which mitigates damage in persisters compared to bacteria that succumb to treatment. However, this paradigm is not true for all persisters. Previous studies show that during treatment with topoisomerase-inhibiting fluoroquinolones (FQs), *Escherichia coli* persisters originating from slow-growing cultures experience antibiotic-induced DNA damage that is indistinguishable from that of non-persisters. *E. coli* persisters require RecA to mediate DNA repair and trigger the SOS response during recovery after FQ treatment, but not prior to or during treatment. Building on this foundation, we examined the role of *recA* in the survival of *Staphylococcus aureus* FQ persisters. We investigated whether the need for *recA* and the importance of the timing of *recA* expression are conserved in *S. aureus* persisters. We found that disabling *recA* through transposon mutagenesis reduces persister survival. Using genetic and single-cell approaches, we discovered that persisters actively express *recA* during the recovery phase. These results underscore the critical timing of DNA repair post-FQ treatment for *S. aureus* persister survival and suggest that targeting DNA damage repair systems could serve as a viable strategy for combating persisters.

25. Rafael Ricci de Azevedo

Mechanism of Shiga toxin-mediated cell death

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Infections by bacteria-expressing Shiga toxin such as enterohemorrhagic Escherichia coli (EHEC) can cause life-threatening conditions and systemic sequelae, especially in children. Although it is well established that Shiga toxin (Stx) can induce acute renal failure syndrome (hemolytic uremic syndrome, HUS) and eventually central nervous system abnormalities, the mechanisms of how the toxin causes damage to these tissues remain to be defined. Therefore, this work aims to determine the molecular determinants and the precise nature of Stx-mediated cell death in human cells. It is known that ribotoxic stress response (RSR) induced by Stx results in caspase-8-dependent activation of caspase-3, and since caspase-3 is traditionally associated with apoptosis, Stx was believed to kill cells via apoptosis. However, recent studies demonstrated that in cells that express high levels of gasdermin E (GSDME), caspase-3 cleaves GSDME, thereby switching the cell death to pyroptosis. By employing the CRISPR-Cas9 gene editing we deleted caspase-3 and GSDME in multiple Stx-sensitive cell types to test if Stx-induced cell death is pyroptosis. Remarkably, genetic deletion of

caspase-3 and GSDME abolished Stx-triggered cell death confirming that Stx indeed induces caspase-3-GSDME-mediated pyroptosis. Since RSR is known to activate the NLRP1 inflammasome as well, we tested the role of NLRP1 in Stx-mediated cell death by deleting NLRP1. Intriguingly, we found that while not absolutely essential, NLRP1 promotes the activation of caspase-8, caspase-3, GSDME, and pyroptosis in response to Stx. Overall, our data show that Stx activates a casp8-casp3-GSDME-dependent pyroptotic pathway in susceptible human cells and NLRP1, when present, functions as an amplifier of this pathway. In summary, we have redefined Stx-mediated cell death in human cells as pyroptosis, a highly inflammatory form of cell death. This finding unravels the potential of GSDME inhibitors as a new therapeutic strategy for aiding patients infected with Shiga toxin-expressing bacteria.

26. Corie M. Owen

Investigating the role of the luteinizing hormone receptor in theca cells during ovulation

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Mammalian oocytes are maintained in individual units known as ovarian follicles. These follicles consist of the oocyte and somatic cells called granulosa cells within a basal lamina, with a theca layer comprised of steroidogenic cells, myofibroblasts, and vasculature directly outside of the basal lamina. Each reproductive cycle, one or more oocytes depending on the species, is ovulated in response to luteinizing hormone (LH). LH binds to its receptor, the luteinizing hormone receptor (LHR), to induce a signal cascade that expels the oocyte from the follicle. Using a mouse with a hemagglutinin tag on the endogenous LH receptor, we have determined that LHR is expressed on a subset of outer mural granulosa cells and theca cells in the ovary. Most ovulation studies focus on the role of LHR signaling in the granulosa cells and the paracrine effects of granulosa LHR signaling on the theca and vasculature surrounding the follicle. However, since LHRs are also present in the theca region, and since blood flow and vascular permeability increase within 10 minutes of LH stimulation, theca LHR signaling may also be directly stimulating ovulatory changes. The first goal of this project is to identify the cells in the theca that express LHR by immunolabelling with known cell markers. The second goal is to determine if the theca LHRs contribute to ovulation by generating a granulosa-specific LHR knockout mouse and investigating changes in vasculature and follicular structure in the time between LH stimulation and ovulation. Preliminary results suggest that LHR expressing cells in the theca have a close association with vasculature, supporting the hypothesis that they may directly contribute to the acute vascular changes observed after LH stimulation. Overall, this project will broaden our understanding of LHR signaling and the role of the theca cells in ovulation.

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