



# IOWA

Center for Biocatalysis  
and Bioprocessing

## 33<sup>rd</sup> Annual CBB Conference Program

# 20 26

# Annual Conference Program

## 2026

07:30 – 08:15 **Reception and Continental Breakfast**

08:15 – 08:35 **Welcome and Introductions**

*Carl Ross Presiding*  
**Director of Development**  
CBB, University of Iowa

**Michael Schnieders**

**Professor and Academic Director**  
CBB, University of Iowa

**Jon Darsee**

**Chief Innovation Officer**  
University of Iowa

08:35 – 09:15

**Kevin Moore**

**Chief Executive Officer**  
NSF RuralSTAMINA Biomanufacturing Engine  
Iowa State University

09:15 – 09:55

**Michael Collingwood**

**Senior Manager of GWE Product Development**  
Integrated DNA Technologies

09:55 – 10:10

Break

10:10 – 10:50

**Eden Tesfu**

**Head of Protein Sciences**  
Bayer Crop Science | St. Louis ACS Local Section Chair

10:50 – 11:30

**Balaji Narasimhan**

**Anson Marston Distinguished Professor and Vlasta Klima Balloun Faculty Chair**  
Iowa State University

11:30 – 11:40

Relocation

11:40 – 12:20

**Lunch and Poster Session A**

12:20 – 01:00

**Lunch and Poster Session B**

01:00 – 01:10

Relocation

*Maria Spies Presiding*

**Professor of Biochemistry and Radiation Oncology**  
University of Iowa

01:10 – 01:50

**Paolo Beuzer**

**Director of Translational Biomarkers**  
CHDI Foundation

01:50 – 02:30

**James Byrne**

**Associate Professor, Radiation Oncology & Biomedical Engineering**  
University of Iowa

02:30 – 02:45

Break

02:45 – 03:05

**Zoe Kramin**

**PhD candidate, Civil & Environmental Engineering**  
University of Iowa

03:05 – 03:25

**Souradip Sinha**

**PhD candidate, Department of Pathology**  
University of Iowa

03:25 – 03:45

**Joseph Correa**

**PhD Candidate, College of Pharmacy**  
University of Iowa

03:45 – 03:55

**Student Poster Awards**  
**Carl Ross**

**Director of Development**  
CBB, University of Iowa

03:55 – 04:00

**Final Comments**  
**Michael Schnieders**

**Professor and Academic Director**  
CBB, University of Iowa

# Invited Speakers



## **Kevin Moore, Chief Executive Officer**

NSF RuralISTAMINA Biomanufacturing Engine  
Iowa State University

## **Biography**

Kevin Moore is Chief Executive Officer of the NSF RuralISTAMINA Biomanufacturing Engine in Iowa and Nebraska, where he works at the intersection of research, industry, and economic development to accelerate commercialization and strengthen the nation's advanced biomanufacturing ecosystem. Previously Chief Technology Officer for Biobased Products at Iowa State University, Moore brings more than 30 years of executive experience in biobased products and renewable chemicals, including leadership roles at Archer Daniels Midland and Trucent Inc, a privately held engineering services and technology company. He has led the commercialization of multiple biobased technologies, including award-winning green chemistry innovations and bioplastics manufacturing. Moore has served as a technical advisor and board member for numerous early-stage biotechnology companies. Moore holds a BS in Chemical Engineering from MIT.

## **Presentation Title and Abstract**

### **NSF RuralISTAMINA Biomanufacturing Engine in Iowa and Nebraska: Building a Rural Bioeconomy Innovation Ecosystem**

Rural communities across Iowa and Nebraska produce some of the nation's most abundant agricultural resources yet capture only a fraction of their economic value. Regional economies remain heavily dependent on a handful of commodity crops and markets with volatile prices and thin margins, while promising innovations face persistent barriers to commercialization, investment, scale-up, and workforce readiness. The NSF RuralISTAMINA Biomanufacturing Engine in Iowa and Nebraska, an NSF Regional Innovation Engine led by Iowa State University, aims to close these gaps by building a lasting advanced biomanufacturing economy across the two-state region.

RuralISTAMINA advances use-inspired research in two technical thrusts: (i) biomanufacturing of pharmaceuticals, biologics, food ingredients, biomaterials and other high-value products, and (ii) biomass conversion of agricultural feedstocks, into industrial materials and chemicals. Cross cutting advances in artificial intelligence, real-time sensing, and automation speed progress in both. Technologies move toward market through an industry-led translation model. The engine supports innovators on opportunities, set up R&D collaborations, and moves technologies through increasing readiness levels. Shared scale-up infrastructure and commercialization resources lower the risk of deployment.

The Engine is organized around four key drivers: use-inspired R&D, translation, workforce development, and regional engagement. On workforce, industry, universities, community colleges, and K-12 educators co-design K-to-Gray curricula and career pathways. Regional engagement uses agricultural and manufacturing extension networks that reach every county in both states, carrying the Engine's impact to a rural population of roughly five million people. By growing biomanufacturing close to the feedstocks that make it possible, RuralISTAMINA aims to create new products, businesses, markets, and skilled jobs in rural communities. It also aims to strengthen domestic supply chains, U.S. competitiveness, and national security. This presentation describes the Engine's vision, goals, cross-sector coalition, and early implementation priorities.



## Biography

Michael is a Senior Manager of Gene Writing and Editing Product Development based in Coralville, Iowa. He leads cross-functional teams focused on developing innovative products and solutions that address customer and business needs. His interests include product innovation, technology development, leadership, and fostering collaboration to drive successful outcomes.

**Micheal Collingwood**, Senior  
Manager of GWE Product  
Development  
Integrated DNA Technologies

## Presentation Title and Abstract

### **The Commercialization Continuum: Moving Research from the Lab to the Market**

Translating scientific discovery into meaningful products requires more than technical innovation. It demands a structured commercialization process that aligns research, product development, manufacturing, and customer needs. This presentation explores the commercialization continuum through the lens of the life sciences industry and highlights how discoveries move from academic laboratories to real-world impact. Drawing on experiences from academia, research, and industry leadership at Integrated DNA Technologies (IDT), the discussion outlines the key stages of product development: opportunity identification, research and development, business case creation, pilot testing, manufacturing scale-up, and commercial launch.

The presentation also examines the role of innovation within a large-scale life sciences organization. Examples include the development of advanced genome editing technologies, with a focus on CRISPR and prime editing platforms. Recent efforts to engineer improved variants and optimize pegRNA designs demonstrate how iterative research and development can enhance editing performance and accelerate the translation of promising technologies into customer-ready solutions.

By examining both the business and scientific dimensions of commercialization, this presentation demonstrates how organizations can bridge the gap between discovery and market impact, ultimately accelerating the delivery of life-changing technologies to researchers, clinicians, and patients worldwide.



## **Eden Tesfu, Head of Protein Sciences**

Bayer Crop Science | St. Louis ACS Local Section Chair

### **Biography**

Dr. Eden Tesfu is a Protein Biochemistry Hub Lead and member of the Plant Biotechnology Leadership Network within Bayer's Crop Science Division. She leads efforts to build and advance protein biochemistry skills and capabilities, including protein and enzyme design, screening, purification, and characterization, to support next-generation agricultural technologies, new trait development, gene editing, and genomics-enabled improvements in seed potential. Her 20-year, multi-industry career spans chemistry, biotechnology, and regulatory strategy. As a strategic leader with experience in discovery science and product development, Dr. Tesfu partners across R&D, Product Supply, and Commercial teams to deliver global business solutions. She is passionate about advancing global food security and agricultural productivity. Before joining Bayer, she held progressively responsible chemistry roles at Pfizer and EAG Laboratories. A recognized leader in her field, Dr. Tesfu values collaboration and partnerships that foster innovation. She serves on the Industry Advisory Board for the University of Missouri–St. Louis Department of Chemistry and Biochemistry and is currently a chair for St. Louis section of American Chemical Society. She holds a B.Sc. in Chemistry from the University of Asmara in Eritrea and M.Sc. and Ph.D. degrees in Organic Chemistry from Washington University in St. Louis. Outside of work, she enjoys cooking and spending time with her family. Dr. Tesfu lives in Chesterfield, Missouri, with her husband and their three children.



**Balaji Narasimhan**, Anson Marston Distinguished Professor and Vlasta Klima Balloun Faculty Chair  
Iowa State University

## **Biography**

Balaji Narasimhan is an Anson Marston Distinguished Professor of Engineering and holds the Vlasta Klima Balloun Faculty Chair in the Department of Chemical and Biological Engineering at Iowa State University (ISU). He has been at ISU since 2001 before which he was on the faculty of the Department of Chemical and Biochemical Engineering at Rutgers University. He served as Associate Dean of Research in the ISU College of Engineering from 2007 to 2013 and oversaw record growths in the college's research portfolio and doctoral degree production. Currently, he directs the Nanovaccine Institute, an interdisciplinary consortium of 87 researchers from 27 universities, national laboratories, research institutes, companies, and hospitals that is focused on the design and development of next generation vaccines and therapeutics. He is a co-founder of a startup called ImmunoNanoMed Inc.

## **Presentation Title and Abstract**

### **Particle-based vaccines: A new paradigm**

The 21st century has already witnessed multiple pandemics. The increased pace of global travel, commerce, and displaced wildlife pose threats that are surpassing control measures. Our over-dependence on antibiotics has led to anti-microbial resistant pathogenic strains. All these factors have created a perfect storm that is severely straining our healthcare. New advances in materials chemistry, nanotechnology, immunology, biomanufacturing, and data and clinical science can be exploited to create next-generation countermeasures. This presentation will focus on novel particle-based vaccine technologies to combat infectious disease.



**Paolo Beuzer**, Associate Professor, Radiation Oncology & Biomedical Engineering  
University of Iowa

## Biography

Paolo Beuzer, PhD is Scientific Director at the CHDI Foundation, a nonprofit organization dedicated to developing treatments for Huntington's disease (HD). After a degree in nanobiotechnology at the University of Trieste, Italy, and a PhD from UPMC Paris Sorbonne, he began his research career at the Salk Institute in San Diego, where he developed novel microscopy tools to visualize single molecules inside living cells. He then moved into drug discovery at Vertex Pharmaceuticals, leading RNA therapy and gene editing programs, before joining CHDI to bridge fundamental biology and clinical application. At CHDI, he leads efforts to understand how errors in DNA repair drive disease progression in HD and to translate these insights into clinical biomarker assays — bringing scientists one step closer to therapies that can slow or prevent the disease.

## Presentation Title and Abstract

### **Curiosity First, Impact Later: A 15-Year Journey from Basic Research to Drug Discovery and Clinical Biomarkers"**

Fundamental research occasionally yields discoveries that, years later, find unexpected large-scale impact and clinical application. This work traces one such journey — from early findings on DNA repair and replication using a classic bacterial gene regulation system, to a novel biomarker assay with real potential to change how we develop drugs for Huntington's disease (HD) and related conditions.

HD is a devastating inherited neurodegenerative disease caused by an expanded stretch of repetitive DNA — CAG repeats — in the huntingtin gene. This mutation continues to expand in specific vulnerable cells, such as neurons, over a patient's lifetime. This ongoing, progressive expansion — called somatic instability — is a key driver of disease onset and progression, and blocking it has become a major therapeutic target pursued by multiple pharmaceutical companies.

The challenge is that somatic instability is a slow process. Detecting whether a drug is actually slowing this process in a clinical trial requires years of follow-up, making it extremely difficult and expensive to evaluate drug candidates.

The prevailing model assumed that DNA repair engagement at the disease-causing region of the huntingtin gene was rare, and that when it did occur, it most often resulted in a mutation — a net change in repeat length. Our findings challenge this picture. Repair events are in fact frequent, while repeat length changes are relatively rare, meaning most repair outcomes are error-free, with no net change to the DNA sequence.

Building on this, we developed URSA (Unscheduled Repair Synthesis Assay), which directly measures DNA repair activity at the mutant huntingtin gene. Because URSA captures all repair events — not just the rare ones that result in a length change — it generates a robust signal in patient blood cells within days rather than years. This makes URSA a promising tool to tell us whether a drug is having its intended effect — and to support and accelerate clinical trials for HD and potentially other repeat expansion diseases and DNA repair-associated cancers.



**James D. Byrne**, Associate Professor, Radiation Oncology & Biomedical Engineering  
University of Iowa

## Biography

James D. Byrne, M.D., Ph.D., is an Associate Professor of Radiation Oncology and Biomedical Engineering at the University of Iowa and a member of Holden Comprehensive Cancer Center. A physician-scientist and biomedical engineer, his research focuses on developing biomaterials, drug-delivery systems, and engineered therapeutic platforms to improve cancer treatment and reduce treatment-related toxicity. His laboratory has pioneered technologies for localized delivery of therapeutic gases, tumor oxygenation, RNA-based radioprotection, and controlled drug delivery, with an emphasis on translating new technologies from the laboratory toward clinical use. Dr. Byrne received his M.D. and Ph.D. from the University of North Carolina and completed residency training through the Harvard Radiation Oncology Program and postdoctoral research at MIT.

## Presentation Title and Abstract

### Cancer Engineering: New Materials for Localized Therapy

The development of innovative biomaterials and drug delivery systems offers new opportunities to overcome longstanding challenges in oncology, including therapeutic resistance, systemic toxicity, and inadequate delivery of treatments to diseased tissues. Our laboratory integrates principles of biomedical engineering, materials science, chemistry, and translational medicine to develop technologies that improve treatment outcomes while reducing adverse effects. A major focus of our research is the development of gas-entrapping materials (GeMs), a versatile biomaterial platform capable of encapsulating and delivering therapeutic gases to targeted tissues. Using biocompatible polymers and scalable fabrication approaches, we have engineered formulations that enable controlled delivery of oxygen and carbon monoxide for applications in cancer treatment, tissue regeneration, and wound healing. Oxygen-entrapping materials alleviate tumor hypoxia, a major contributor to resistance to radiotherapy and immunotherapy, by increasing intratumoral oxygen availability and enhancing treatment response. Carbon monoxide-entrapping materials exploit the biological signaling properties of carbon monoxide to modulate inflammation, promote tissue repair, and enhance antitumor immunity. These technologies demonstrate how the controlled delivery of small gaseous molecules can produce meaningful biological effects across diverse disease settings. Collectively, our work illustrates how interdisciplinary engineering strategies can transform simple materials and biologically active molecules into innovative therapeutic platforms. By integrating biomaterials design, controlled-release engineering, mechanistic biological studies, and clinically relevant models, we aim to accelerate the translation of scalable, accessible technologies that improve patient outcomes across oncology and regenerative medicine.



## Biography

Zoe Kramin is a PhD candidate in the Civil & Environmental Engineering department under the mentorship of Dr. Craig Just, in association with the Wastewater to Energy Program and IIHR-Hydrosience & Engineering. Her research focuses on advancing anaerobic digestion and wastewater treatment through molecular biology, machine learning, and real-time monitoring technologies.

**Zoe Kramin**, PhD candidate,  
Civil & Environmental  
Engineering  
University of Iowa

## Presentation Title and Abstract

### **Predicting anaerobic digestion stability with genetic early-warning indicators**

Anaerobic digestion is a biological process that converts organic wastes into biogas, a renewable energy source primarily composed of methane. Although anaerobic digesters can reliably process organic wastes, sudden changes in waste composition or large increases in organic loading can disrupt the microbial community responsible for methane production and ultimately lead to digester failure. Understanding how individual methanogens respond to these disturbances could improve the ability to monitor and maintain digester stability. Methanogens are a specialized group of microorganisms responsible for the final step of methane production in anaerobic digestion. Despite their central role, relatively little is known about how methanogen activity changes at the genetic level in response to organic shocks. In particular, identifying microbial responses that occur before typical process failure parameters could provide valuable indicators of digester instability.

This study investigates the expression of *mcrA*, a gene encoding methyl-coenzyme M reductase, a key enzyme in methanogenesis, across 37 methanogen species during organic shock conditions. Changes in *mcrA* expression were evaluated to characterize species-specific responses to high organic loading with varying compositions. Preliminary data indicate that the novel application of a custom gene panel successfully quantified *mcrA* expression from 7 methanogenic genera. Of the quantified genera, a stability-indicator genus, *Methanothrix*, was dominant in Iowa City digestate. The *mcrA* expression of *Methanothrix* species decreased two hours after organic loading spikes of protein and carbohydrate, suggesting rapid inhibition of methanogenic activity. In contrast, the *mcrA* expression of instability-indicator genus, *Methanosarcina*, did not decrease during the initial stages of organic stress.

These contrasting responses suggest that methanogen-specific *mcrA* expression may provide an early biological indicator of anaerobic digester stability. Characterizing these responses may improve understanding of microbial metabolic responses to process disturbances and ultimately support improved monitoring and control of anaerobic digestion systems.



**Souradip Sinha**, PhD candidate,  
Department of Pathology  
University of Iowa

## Biography

Souradip Sinha is a PhD candidate in the Department of Pathology at the University of Iowa in the laboratory of Dr. Munir Tanas. His doctoral research focuses on epigenetic mechanisms in sarcomas, particularly the role of the ATAC histone acetyltransferase complex as an oncogenic driver in fusion protein-positive and -negative sarcomas.

Originally from Kolkata, India, he earned his bachelor's degree from Scottish Church College, University of Calcutta, and a master's degree in Biotechnology from Jadavpur University. At the University of Iowa, he joined the Biomedical Science Program in 2021 and the Molecular Medicine Program in 2022. As a Center for Biocatalysis and Bioprocessing (CBB) Fellow (2023–2025), he obtained an industry-focused internship with the University of Iowa Pharmaceuticals (UIP), where he worked with multidisciplinary teams within a CDMO facility.

## Presentation Title and Abstract

### **The ATAC histone acetyltransferase complex promotes oncogenic growth and transcriptional programs in sarcomas**

The ADA2A-containing (ATAC) histone acetyltransferase (HAT) complex regulates chromatin accessibility and gene transcription and has emerged as an important oncogenic chromatin regulator. The complex consists of a YEATS2/ZZZ3-containing core module and a catalytic HAT module containing either GCN5 or PCAF. Our laboratory previously demonstrated that the ATAC complex is recruited by TAZ/YAP fusion oncoproteins that drive epithelioid hemangioendothelioma (EHE). However, its role in non-EHE sarcomas remains poorly understood.

siRNA/shRNA-mediated knockdowns of YEATS2, ZZZ3, GCN5, PCAF, and E2F3 were performed in EHE and fusion-negative sarcoma models. Functional consequences were evaluated using immunoblotting, proliferation assays, soft-agar colony-formation assays, and xenograft studies. TurboID proximity-labeling and co-immunoprecipitation were used to identify ATAC complex-associated proteins. Transcriptomic programs associated with YEATS2/ZZZ3 expression were analyzed using TCGA-SARC datasets. Therapeutic vulnerability was assessed using GSK4027(GCN5/PCAF-inhibitor) and palbociclib(CDK4/6 inhibitor).

YEATS2 and ZZZ3 were required to maintain ATAC complex-dependent H3K9 acetylation across sarcoma models. Genetic depletion of YEATS2 suppressed proliferation, anchorage-independent growth, and xenograft tumor growth. Rescue and domain-mapping studies demonstrated that intact YEATS2 and ZZZ3 architecture is necessary for ATAC complex localization and HAT activity. Proteomic analyses identified E2F3 as a transcription factor interacting with the ATAC complex, and E2F3 depletion phenocopied ATAC complex disruption. Depletion of either E2F3 or YEATS2 reduced expression of the canonical E2F-target genes CDC6 and MCM3. Bioinformatic analyses demonstrated that elevated ATAC complex expression is associated with E2F-driven cell-cycle, DNA replication, and DNA repair programs. Combined inhibition of ATAC complex and CDK4/6 signaling produced synergistic growth suppression.

Conclusions: The ATAC complex represents a critical epigenetic dependency in sarcomas. We identify an E2F3–ATAC transcriptional axis that promotes oncogenic growth through cell-cycle and genome maintenance programs. Furthermore, ATAC complex activity represents a therapeutically actionable vulnerability, and combined targeting of ATAC complex and CDK4/6 signaling may represent a promising therapeutic strategy for sarcomas.



**Joseph Correa, PhD**  
Candidate, College of  
Pharmacy  
University of Iowa

## Biography

Joseph Correa is a Pharmaceutics PhD candidate in the University of Iowa College of Pharmacy, where he conducts research in the laboratory of Dr. Nicole Brogden. His doctoral research focuses on formulating dissolving microneedles for transdermal epinephrine delivery in cases of acute anaphylaxis, with the goal of developing easily administered therapies that can help ease patient burdens. Joseph earned his BS in Biology and BA in Biochemistry from the University of Northern Iowa in 2023. He joined the University of Iowa College of Pharmacy to pursue graduate studies in Pharmaceutics and is a Center for Biocatalysis and Bioprocessing (CBB) Fellow (2024–2026).

His research interests are driven by a passion for developing accessible drug delivery technologies and improving patient experiences through innovative therapeutic approaches.

## Presentation Title and Abstract

### Rapidly Dissolving Microneedles for Acute Anaphylaxis

Epinephrine is the first-line treatment for acute anaphylaxis. Typically delivered with autoinjectors, it has a rapid onset of action and reaches peak efficacy within ten minutes. However, with a half-life less than five minutes, efficacy can be short-lived, which often requires multiple doses before medical care arrives. Transdermal drug delivery systems offer painless administration, customizable release profiles, and patient favorability over injections. To limit the need for additional doses while still providing fast relief, we are developing polymeric dissolving microneedles (dMNs) for painless and rapid epinephrine delivery with continued release.

In our preliminary studies we screened polymers based on epinephrine release, dMN forming ability, and epinephrine permeation across porcine skin. Of the polymers investigated, pullulan (PL) and polyvinyl pyrrolidone (PVP) had the highest epinephrine release, releasing up to 63% of their loading in 30 minutes. PL dMNs showed superior mechanical strength, withstanding 20 N of force without breaking. In permeation studies, 10% (w/w) PL with 2% (w/w) epinephrine demonstrated that dMNs improved permeation versus intact skin controls. However, quantifiable epinephrine concentrations were not detected until 30 minutes, which may be attributed to the dMN dissolution rate. To overcome this limitation, we are optimizing the dMN formulation to achieve >90% dissolution within 60 seconds, thus allowing earlier epinephrine permeation. We screened dMNs fabricated with various polymers including PVP, hydroxypropyl methylcellulose (HPMC), and polyvinyl alcohol (PVA) and evaluated the rate and extent of dissolution after insertion into porcine skin.

15% and 30% (w/w) PVA formed strong dMN arrays that withstood breaking upon insertion but demonstrated no dissolution within 60 seconds. HPMC failed to produce sufficient arrays for further testing. 30% (w/w) PVP arrays, however, withstood breaking and achieved >80% dissolution within 30 seconds. The mechanical properties of the arrays depended heavily on preparation techniques, with small adjustments correlating with large changes in mechanical strength. Future studies will investigate the correlation of dMN dissolution rate and epinephrine permeation and will evaluate the effect of localized warming on epinephrine permeation.

## Poster Presentations

| Poster No. | Presenter           | Poster Title                                                                                                                                      |
|------------|---------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| 1          | Esraa Mohamed       | <i>Pulmonary and Genomic Toxicity Induced by Phosphine Gas Exposure: Insights from a Mouse Model toward Therapeutic Target Identification</i>     |
| 2          | Min Chen            | <i>Context-Dependent Protective Effect of Polysorbate 80 on Monoclonal Antibody Particle Formation</i>                                            |
| 3          | Nafis Md Irfan      | <i>IL-1 alpha expression induces drug resistance to all generations of EGFR TKIs in head and neck squamous cell carcinoma</i>                     |
| 4          | Tyler Woodward      | <i>The Oligomeric State of Rad6-Rad18 and Its Interaction with TLS Proteins</i>                                                                   |
| 5          | Edozie Okpara       | <i>Investigating the cell-type-specific effects of miR29a-KLF4 axis and miR29a-ADAM12 signaling in Diabetic Peripheral Arterial Disease (PAD)</i> |
| 6          | Avelin Thepsomphone | <i>Elucidating the Mechanism of Action of trans-Banglene</i>                                                                                      |
| 7          | Samuel Yu           | <i>Investigating Upstream Regulators of PI3K–Hippo Pathways in Sarcoma</i>                                                                        |
| 8          | Isha Muzumdar       | <i>Evaluating T-Cell Morphology Under Handling Stress Using Flow Imaging Microscopy.</i>                                                          |
| 9          | Hossein Zare        | <i>A Modular Model for Bacterial Biofilm Dispersion</i>                                                                                           |
| 10         | Ramkrishna Sen      | <i>Actively targeting colorectal cancer using nanoparticles co-loaded with small molecule inhibitors</i>                                          |
| 11         | Lilly Duff          | <i>Single molecule mechanics reveals convergent allostery in glutamate racemase</i>                                                               |

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|-----------|----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| <b>12</b> | <b>Rose Gogal</b>                | <i>The prevalence of protein misfolding as a mechanism for hereditary deafness</i>                                                    |
| <b>13</b> | <b>Anna Claire</b>               | <i>Muscle phosphofructokinase as a potential link between mechanics, metabolism and gene expression</i>                               |
| <b>14</b> | <b>Luke Handlos</b>              | <i>Oligomeric States and Conformational Flexibility of the DNA Damage Bypass Complex Rad6-Rad18</i>                                   |
| <b>15</b> | <b>Edozie Samuel Okpara</b>      | <i>Investigating the Cell-Type-Specific Effects of miR29a-KLF4 and miR29a-ADAM12 signaling in Peripheral Arterial Disease (PAD)</i>   |
| <b>16</b> | <b>JoJo Peplinski</b>            | <i>Investigating Stromal-Modulating Agents to Alter Tumor Mechanics and Enhance Drug Delivery in Pancreatic Ductal Adenocarcinoma</i> |
| <b>17</b> | <b>Rachel Kehrberg</b>           | <i>Fatty Acid Oxidation Restrains Mechanically Induced Junctional Reinforcement Through Effects on Membrane Lipid Composition</i>     |
| <b>18</b> | <b>Atonu Chakraborty</b>         | <i>Temporal Remodeling of ABC Transporter Programs During Acquired Paclitaxel Resistance in Ovarian Cancer</i>                        |
| <b>19</b> | <b>Joseph Correa</b>             | <i>Rapidly Dissolving Microneedles for Acute Anaphylaxis</i>                                                                          |
| <b>20</b> | <b>Andrew Thiel</b>              | <i>Polarizable Constant pH Molecular Dynamics for Protein-Cofactor and Protein-DNA Complexes</i>                                      |
| <b>21</b> | <b>Pornpoj Phruttiwanichakun</b> | <i>Growth Differentiation Factor 5 (GDF5) as a potential therapy for post-traumatic osteoarthritis.</i>                               |
| <b>22</b> | <b>Jake Miller</b>               | <i>Accurate Prediction of Protein-DNA Relative Binding Free Energies Using the AMOEBA Force Field</i>                                 |
| <b>23</b> | <b>Sarah Torrence</b>            | <i>Recombinant Prohibitins Mitigate Pro-Inflammatory Priming Response and Acute Kidney Injury Induced by Lipopolysaccharide</i>       |

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|-----------|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>24</b> | <b>Shweta Wani</b>      | <i>Heparin-Coated PAMAM/CpG G10 Nanoparticles Targeting Plasmacytoid Dendritic Cells Promotes Type I Interferon Mediated Immune Activation In Vitro</i> |
| <b>25</b> | <b>Emily Jansen</b>     | <i>An Engineered Mixed Microbial Culture for Self-Sustained 1,4-Dioxane Bioremediation</i>                                                              |
| <b>26</b> | <b>Elizabeth Walker</b> | <i>Synthetic biology of non-conventional <i>Cupriavidus necator</i> strains through enhanced recombineering for improving PHA production</i>            |
| <b>27</b> | <b>Sophie Granger</b>   | <i>Single-Molecule Mechanics Reveals Convergent Allostery in Glutamate Racemase</i>                                                                     |

**Even numbered posters = Session A      11:40 - 12:20**

**Odd numbered posters = Session B      12:20 - 1:00**

## Poster #1

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| <b>Esraa Mohamed</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Pulmonary and Genomic Toxicity Induced by Phosphine Gas Exposure: Insights from a Mouse Model toward Therapeutic Target Identification |
| <p><b>Background:</b> Phosphine (PH<sub>3</sub>); a highly toxic gas, used in the industry as polymerization initiator, as an intermediate for preparation of some flame retardants, and as a fumigant. Human exposure occurs by inhalation of accidentally released gas or when phosphide-containing pesticides are ingested. It is also a potential chemical threat agent. The mechanism of PH<sub>3</sub> toxicity is not fully understood. There is no antidote available due to the absence of reliable therapeutic targets.</p> <p><b>Objectives:</b> This study examines the pulmonary effects of acute PH<sub>3</sub> inhalation to support future treatment strategies and identifies key biological pathways affected in the lungs and hearts.</p> <p><b>Methods:</b> Female mice were exposed to three PH<sub>3</sub> doses in a dynamic whole-body exposure chamber. Following exposure, respiratory functions, total protein levels in BALF, and lung pathology were evaluated. Bulk RNA sequencing of PH<sub>3</sub>-exposed lungs and hearts was performed and differentially expressed genes were analyzed.</p> <p><b>Results:</b> Animals exposed to high PH<sub>3</sub> doses experienced a significant decrease in respiratory rate. There was a significant increase in total protein and cell counts in BALF of PH<sub>3</sub>-exposed mice and histopathological evaluation of lungs showed foci of hypercellularity and scattered vessels filled with platelet aggregates and neutrophils, indicative of early pulmonary thrombi formation. Gene expression analysis revealed upregulation of genes involved in inflammation, cell cycle and apoptosis. The MAPK pathway was among the most impacted pathways in lung and heart tissues. Fos and Jun were significantly upregulated following exposure, all of which are components of AP-1 protein complex, a potentially key element in inducing injuries subsequent to PH<sub>3</sub> exposure and therefore a promising therapeutic target.</p> <p><b>Conclusion:</b> Our study investigated pulmonary toxicity induced by acute inhalation exposure to PH<sub>3</sub> gas by using a whole-body inhalation exposure model. The results showed that the inhalation exposure to high doses of PH<sub>3</sub> induced marked changes in clinical observations, respiratory dynamics, histopathology, and gene expression, highlighting the lethal effects on the pulmonary and cardiovascular systems. During exposure to high doses, mice experienced a marked decrease in respiratory rate and minute volume, leading to euthanasia within 0.5-5 h postexposure, suggesting that severe tissue and systemic damage developed after exposure. The RNA-seq findings provide a comprehensive transcriptomic characterization of potential molecular responses to exposure and identified potential mechanisms underlying lung and heart tissue injury following exposure. We propose the AP-1 complex as a potential key element in inducing injuries after high PH<sub>3</sub> exposure, implicating AP-1 as a potential therapeutic target in the treatment of PH<sub>3</sub>-induced toxicity in clinical practice.</p> |                                                                                                                                        |

## Poster #2

**Min Chen**

Context-Dependent Protective Effect of Polysorbate 80 on Monoclonal Antibody Particle Formation

**Background:** Monoclonal antibodies (mAbs) are exposed to air-liquid and solid-liquid interfaces during manufacturing, storage, and handling. Mechanical stress can increase interfacial exposure and promote aggregation and subvisible particle formation. Polysorbate 80 (PS80) is widely used to mitigate interfacial damage, but its protective effect may depend on antibody properties, stress type, and surface chemistry. Here, we evaluated how surface hydrophobicity modulates PS80-mediated stabilization of two IgG1 mAbs under shaking and stirring stress.

**Objectives:** Two IgG1 monoclonal antibodies were subjected to shaking and stirring in hydrophilic and hydrophobic glass vials, with and without PS80, to evaluate the effect of surface chemistry and surfactant on particle formation.

**Methods:** mAb1 (1 mg/mL, 20 mM histidine, pH 6.0) and mAb2 (0.5 mg/mL, PBS, pH 7.2) were formulated with or without 0.01% w/v PS80. Samples were stressed by horizontal shaking at 200 rpm for 72 h or magnetic stirring at 300 rpm for 90 min in hydrophilic or DDS-silanized hydrophobic glass vials. Particle formation was characterized using flow imaging microscopy (FIM) and dynamic light scattering (DLS).

**Results:** PS80 reduced mechanically induced particle formation for both mAbs under shaking and stirring conditions. mAb2 was more susceptible to shaking stress than mAb1, generating higher particle counts. The effect of surface hydrophobicity was molecule- and PS80-dependent. Without PS80, mAb1 showed comparable particle formation on hydrophilic and hydrophobic surfaces, whereas with PS80, particle formation was lower on the hydrophobic surface. In contrast, mAb2 showed lower particle formation on the hydrophobic surface without PS80, while little surface-dependent difference was observed in the presence of PS80. DLS results were generally consistent with FIM observations.

Overall, PS80 effectively reduced mechanically induced particle formation, but the magnitude of protection depended on mAb identity and surface chemistry. These findings demonstrate that surface chemistry can modulate surfactant-mediated stabilization in a molecule-dependent

**Conclusion:** PS80 reduced mechanically induced particle formation in both IgG1 mAbs under shaking and stirring conditions, although the magnitude of protection varied with mAb identity and surface chemistry. The effect of surface hydrophobicity was not consistent across the two antibodies and depended on the presence of PS80. These findings suggest that surface chemistry modulates surfactant-mediated stabilization in a molecule-dependent manner.

## Poster #3

**Nafis Md Irfan**

IL-1 alpha expression induces drug resistance to all generations of EGFR TKIs in head and neck squamous cell carcinoma

**Background:** This work is important since it identifies the role of IL1-alpha in mediating inflammation in response to Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors(TKI) in head and neck squamous cell carcinoma (HNSCC). This study provides mechanistic link between therapy induced inflammation that leads to drug resistance. The translational side of the study is to use combination therapy of EGFR TKI with IL1 alpha signaling blockade for improve the efficacy of these drugs.

**Objectives:** We are trying to achieve new targets (IL1 alpha signaling blockade) to reduce inflammation that leads to drug resistance of EGFR TKIs in HNSCC.

**Methods:** I am going to employ in vitro cell viability through MTT assay and measure IL6, IL1 alpha release through ELISA in response to all four generations of EGFR TKI in three head and neck cancer cell line. We will be targeting to block IL1 alpha through neutralizing antibody and IL1 alpha receptor blockade through IL-1 alpha receptor to antagonist to see if it reduces EGFR TKI induced IL-6. Moreover, we will overexpress the IL1 alpha in CAL27 HNSCC cell line to see the EGFR TKI response.

**Results:** We found that all generations of EGFR TKI although inhibits HNSCC cancer growth but surprisingly creates an inflammatory condition led by IL-6, IL1 alpha but not IL1 Beta. Then subsequent blockade of IL1-alpha signaling reduced EGFR TKI induced IL-6. Also, IL1-alpha overexpression in HNSCC leads to EGFR TKI resistance.

**Conclusion:** Epidermal Growth Factor Receptor (EGFR) expression is upregulated in the majority of head and neck squamous cell carcinomas (HNSCC) and is associated with poor clinical outcomes. Unfortunately, the incorporation of EGFR inhibitors (EGFRIs) into the management of HNSCC has not improved long term survival rates in HNSCC patients despite high EGFR expression. Additionally, EGFR tyrosine kinase inhibitors (TKIs) in particular, have failed to show clinical benefit for HNSCC patients in clinical trials. Therefore, the identification and understanding of strategies that will improve the efficacy of EGFRIs may improve patient treatment and survival. Previous work in our lab has shown that HNSCC cells treated with erlotinib, a first-generation EGFR TKI, increased expression and release of the proinflammatory cytokine interleukin-1 alpha (IL-1a) resulting in inflammation and the development of drug resistance. The goal of this study is to determine if IL-1a-induced drug resistance is maintained across second, third and fourth generation EGFR TKIs.

The proposed work has implications both in basic and translation science in cancer biology particularly in the context of HNSCC. This work try to underpin the role of IL-1 alpha expression as a central mediator of poor response and resistance to all EGFR TKIs via an EGFR mutation-independent mechanism. Hence, targeting IL-1a expression/signaling could be a promising strategy to improve long term tumor response to EGFR TKIs in HNSCC.

## Poster #4

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| <b>Tyler Woodward</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | The Oligomeric State of Rad6–Rad18 and Its Interaction with TLS Proteins |
| <p><b>Background:</b> Translesion synthesis (TLS) is a DNA damage tolerance pathway where specialized DNA polymerases are recruited to stalled replication forks. These polymerases, such as pol <math>\eta</math> and Rev1, can catalyze nucleotide incorporation using damaged templating bases. TLS is regulated by the E2–E3 ubiquitin ligase complex known as Rad6–Rad18. The Rad6–Rad18 complex has recently been demonstrated to interact with pol <math>\eta</math>, leading to the hypothesis that the complex is involved in polymerase recruitment. Yet, the exact mechanism remains poorly understood</p> <p><b>Objectives:</b> We seek to understand the role of Rad6–Rad18 on polymerase recruitment during TLS.</p> <p><b>Methods:</b> We utilized yeast two–hybrid assays, mass photometry, biolayer interferometry, and yeast survival assays.</p> <p><b>Results:</b> We found that the oligomeric state of Rad6–Rad18 regulates its interactions with TLS polymerases. Mainly, we found that higher–order species of Rad6–Rad18 are capable of binding pol <math>\eta</math> but not Rev1. On the other hand, we found that Rad18 has auto–inhibition for Rev1 binding which is relieved when Rad6–Rad18 complexes dissociate into free Rad18 protomers.</p> <p><b>Conclusion:</b> Our data support a sequential recruitment model in which Rad18 auto–inhibition preferentially recruits Pol <math>\eta</math> to the Rad6–Rad18 complex at stalled replication forks. Subsequent dissociation of Pol <math>\eta</math> and Rad6 is proposed to relieve autoinhibition, allowing Rev1 to bind. This ordered mechanism may reduce mutagenesis by favoring the less error–prone Pol <math>\eta</math> before recruitment of Rev1. Additional work is needed to understand the cause of the dissociation of the pol <math>\eta</math> and Rad6</p> |                                                                          |

## Poster #5

### Edozie Okpara

Investigating the cell-type-specific effects of miR29a-KLF4 axis and miR29a-ADAM12 signaling in Diabetic Peripheral Arterial Disease (PAD)

**Background:** Peripheral Artery Disease (PAD) is a serious health condition. It happens when narrowed leg arteries block vital blood flow. The disease impacts over 200 million people worldwide and nearly 12 million Americans. Patients suffer from severe leg pain, trouble walking, and open wounds. In the worst cases, it leads to leg amputations. Patients with diabetes face even worse outcomes. Their bodies struggle severely to repair damaged leg muscles and blood vessels. The exact reason for this healing failure is still unknown. What is the proposed solution? Our team discovered a specific, tiny "control switch" in the body. This switch blocks natural healing. In diabetes and low blood flow conditions, this switch turns on. It actively stops muscles and blood vessels from repairing themselves. This project will test if blocking or removing this switch can restore the body's natural ability to heal. We will study cells in environments that imitate diabetes. We will also use specialized models to turn this switch off in specific leg tissues. What is the impact on public health? This research will show exactly how diabetes controls blood flow to the leg and prevents it from properly healing after injury. By understanding this process, we can help scientists develop targeted, life-saving therapies. These treatments will regrow healthy blood vessels and repair damaged muscles. Ultimately, this work aims to help restore normal walking ability. It will prevent painful leg losses and improve the lives of millions of diabetic patients fighting PAD.

#### **Objectives:**

1. Determine whether ischemia, diabetes and combined diabetic-ischemic stress alter the miR-29a-KLF4 and miR-29a-ADAM12 signaling axes, in mouse-derived myoblasts (MBs) and Endothelial Cells (ECs) and identify additional miR-29a targets
2. Determine the effects of cell-specific miR-29a knockout in experimental PAD in diabetic and non-diabetic settings.
3. Determine whether targeted inhibition of skeletal muscle miR-29a improves ischemic muscle health in DM PAD.

**Methods:** I have summarized the seven (7) major methods/techniques I will use for my project below:

1. Taqman and Sybr green qPCR for gene expression studies.
2. Ponceau Western Blot to validate results from expression studies
3. Mice conditional deletion models to set up cell-specific target miRNA deletion
4. miRNAScope in situ hybridization studies to visualize miR-29a deletion in two cell types being studied.
5. Flow cytometry to establish cell-type percentage purity after (ex vivo) isolation
6. Longitudinal Blood Flow measurements using lab's Laser Scatter Contrast imaging device.
7. In vitro culturing of endothelial cells, myoblasts and myofibres

**Results:** My preliminary data demonstrate that miR-29a, KLF4 (Krupel-like factor-4) and ADAM12 (a disintegrin and metalloproteinase-12) are expressed and ischemia-responsive in skeletal muscle ECs and MBs, with distinct cell type-dependent ischemic regulation. However, it remains to be seen how DM stress alone and DM + ischemic stress will impact the expression trends I have observed so far.

**Conclusion:** Despite the disproportionate burden of PAD in patients with diabetes, the molecular mechanisms underlying impaired vascular and skeletal muscle regeneration remain poorly defined. Preliminary data identify miR-29a and its downstream targets, KLF4 and ADAM12, as ischemia-responsive regulators in skeletal muscle endothelial cells and myoblasts, supporting a novel role for this pathway in tissue repair. This project will establish the cell-specific contribution of miR-29a to diabetic ischemic injury and evaluate its therapeutic potential using genetic and targeted inhibition approaches. The findings are expected to advance our understanding of diabetic PAD pathogenesis and provide a foundation for developing regenerative therapies aimed at improving limb salvage and functional recovery.

## Poster #6

### Avelin Thepsomphone

#### Elucidating the Mechanism of Action of trans-Banglene

**Background:** Despite significant therapeutic advances, neurodegenerative diseases remain untreatable, and diagnosis rates continue to rise. Many neurodegenerative diseases are characterized by the accumulation of cytotoxic protein aggregates that are found to be co-localized with high concentrations of iron (Ndayisaba et al.). High levels of ferrous iron trigger ferroptosis--a form of programmed cell death through the accumulation of lipid peroxides in the cell membrane (Zhou et al.). In neurodegenerative diseases, lipid peroxidation and iron dyshomeostasis are prevalent and indicate iron plays a notable role in the progression of these diseases.

**Objectives:** trans-Banglene (t-BG) is a small neurotrophic molecule that has previously demonstrated significant alterations in iron-binding proteins in PC-12 cells by the Williams research group. Intracellular iron measurements demonstrated lower ferrous iron in t-BG treated cells but not in Nerve Growth Factor (NGF) treated cells (NGF is a native neurotrophic protein). Significant differences in Fto, Ferritin, and Hmox1 suggest t-BG alters iron-binding proteins to a greater extent than NGF alone. This led to the hypothesis that t-BG modulates iron homeostasis in our model neuronal cell line PC-12. A cellular target and mechanism of action remain unknown for t-BG thus the goal of this research is to gain preliminary data and conclusively annotate a mechanism of action to understand how t-BG impacts classic neurotrophic responses and iron modulation.

**Methods:** To identify the recognition/binding targets of t-BG in the cell, a photolabile cross-linking assay using a modified derivative of t-BG has been designed and synthesized. Neuritogenesis assays will be performed to confirm the photolabile cross-linking derivative retains neuritogenic activity. Living cells will be treated with this compound and irradiated to allow photo-affinity labeling studies for identification of nearby molecules/proteins and to gain target localization information.

**Results:** To date, optimizations on the neuritogenesis assays have been performed to discover optimal imaging conditions and MTT assays have been performed to the study the effects of t-BG in the presence of oxidative stress on PC-12 cells. t-BG appears to have an effect on PC-12 cells in the presence of oxidative stress, but results remain inconclusive.

**Conclusion:** trans-Banglene can address the pharmacokinetic limitations that some native neurotrophins suffer from, such as oral bioavailability and blood brain permeability. Therefore it can be further developed as a potent biomedical tool and serve as a platform for drug development to treat neurodegenerative diseases. To evaluate this potential, the mechanism of action for trans-Banglene must be elucidated.

## Poster #7

**Samuel Yu**

Investigating Upstream Regulators of PI3K–Hippo Pathways in Sarcoma

**Background:** Sarcomas are a heterogeneous group of cancers with limited effective targeted therapies. PI3K signaling is frequently dysregulated in sarcomas, often through loss of PTEN, and promotes tumor growth through both the canonical Akt–mTORC1 pathway and a parallel TAZ/YAP–TEAD transcriptional program. TAZ/YAP are transcriptional co-activators that drive oncogenic gene expression and are regulated by the Hippo pathway consisting of the core kinases MST1/2 and LATS1/2. PI3K signaling regulates TAZ/YAP activity through LATS1/2-dependent mechanisms, suggesting functional convergence between PI3K and Hippo signaling. Emerging evidence suggests that epigenetic silencing of upstream Hippo pathway regulators, including MST1/2 and MAP4K family kinases, contributes to aberrant TAZ/YAP activation in sarcoma.

**Objectives:** This study aims to investigate whether HDAC inhibition can restore expression of upstream Hippo pathway components and suppress YAP/TAZ signaling, while also evaluating the potential for combination strategies targeting the PI3K–Hippo signaling network, given the convergence of these pathways on tumor-promoting transcriptional and translational programs.

**Methods:** RD (embryonal rhabdomyosarcoma) cells were treated with romidepsin (HDAC inhibitor) and everolimus (mTORC1 inhibitor), alone or in combination. Cell proliferation was assessed using CCK-8 assays, clonogenic outgrowth was used to evaluate long-term survival, and western blotting was performed to assess expression of Hippo pathway components and downstream signaling proteins.

**Results:** Romidepsin treatment significantly reduced proliferation (>90%) and clonogenic outgrowth (>90%) in RD cells at low nanomolar concentrations. These effects were accompanied by increased expression of the upstream Hippo pathway regulators MST1 and MAP4K4/6 and reduced total YAP protein levels. Changes in YAP/TAZ phosphorylation were also observed, whereas total TAZ expression remained largely unchanged, suggesting broader remodeling of Hippo pathway signaling following HDAC inhibition. Together, these findings support a model in which HDAC inhibition alleviates epigenetic suppression of upstream Hippo pathway regulators and reduces YAP/TAZ signaling.

To evaluate therapeutic opportunities arising from PI3K–Hippo pathway convergence, RD cells were treated with romidepsin and everolimus alone or in combination. Combination treatment resulted in greater suppression of proliferation and clonogenic outgrowth than either single-agent treatment, supporting the therapeutic potential of coordinated Hippo pathway reactivation and PI3K pathway inhibition.

**Conclusion:** Our findings identify epigenetic regulation of the Hippo pathway as a potential therapeutic vulnerability in sarcoma. HDAC inhibition increased expression of upstream Hippo signaling components and was associated with reduced YAP/TAZ signaling, decreased proliferation, and impaired clonogenic survival. These findings provide a rationale for therapeutic strategies that combine Hippo pathway reactivation with PI3K pathway inhibition and support further mechanistic and in vivo studies to evaluate their clinical potential in sarcoma, including investigation of the dose- and time-dependent effects of romidepsin on Hippo pathway signaling.

## Poster #8

Isha Muzumdar

### Evaluating T-Cell Morphology Under Handling Stress Using Flow Imaging Microscopy.

**Background:** Cell based medicinal products (CBMPs) including T-cell based products are increasingly being considered for treating various diseases. T-cell products are often administered via intravenous infusions. However, syringes may be used during the preparation, sampling, transfer or administration exposing cells to mechanical forces such as shear stress. Although syringe handling effects have been studied extensively for protein pharmaceuticals, their effects on T-cell morphology, viability and quality are poorly understood.

**Objectives:** This study employs flow imaging microscopy (FIM) to investigate the effects of syringe stress, silicone oil and flow rates on T-cell morphology.

**Methods:** Primary T-cells were isolated from PBMCs and suspended in 1X Dulbecco's Phosphate Buffered Saline (D-PBS) to obtain concentration of  $1 \times 10^6$  cells/mL. Unless otherwise specified, 1.0 mL volume of T-cell suspension was manually drawn into 3.0 mL siliconized disposable syringe fitted with an 18G hypodermic needle and loaded onto a syringe pump. These samples were evaluated in triplicate under two experimental conditions.

The first condition involved varying flow rates. The syringe plunger was withdrawn at flow rates of either 9 mL/min and 300  $\mu$ L/min. For the second experimental condition, samples were manually drawn into either siliconized disposable or non-siliconized syringes. The samples were subjected to either single or triple reciprocation cycle. The final stressed sample was collected in a microcentrifuge tube. The unstressed controls and stressed samples were analyzed using FlowCam® 8000.

**Results:** FIM showed that the proportion of cells in 0.9-1.0 symmetry interval was significantly higher in 9 mL/min post-needle sample (26.00 %  $\pm$  4.69 %) than 300  $\mu$ L/min post-needle sample (18.52 %  $\pm$  1.96 %). Syringe siliconization affected symmetry and aspect ratio with non-siliconized syringes having lower proportion of cells at higher morphology intervals.

**Conclusion:** Flow rate, duration of stress exposure and syringe siliconization produced small but statistically significant (p-value  $\leq$  0.05) changes in T-cell symmetry and aspect ratio without affecting T-cell diameter.

## Poster #9

Hossein Zare

### A Modular Model for Bacterial Biofilm Dispersion

**Background:** Localized thermal treatment can significantly decrease bacterial populations on an infected implant associated surface, but surface reduction may not represent treatment success. In mature *Pseudomonas aeruginosa* PAO1 biofilms, a large fraction of the surface population can rapidly enter the surrounding media even before the thermal treatment starts. Some bacterial cells may survive, migrate through adjacent material, and later recolonize the treated surface. The treatment problem therefore is following biofilm release, heat transfer, spatial transport, bacterial survival, and delayed recovery rather than surface killing alone.

**Objectives:** The main purpose of this presentation is to develop a framework that connects experimentally observed biofilm release and transport with thermal exposure and post-treatment recolonization. The model is designed to distinguish bacterial loss from redistribution and to identify measurable treatment outcomes beyond surface CFU reduction.

**Methods:** A five-module framework was constructed from PAO1 experiments and supporting literature: (I) rapid surface release and biofilm–planktonic exchange; (II) heat delivery through PVA tissue phantoms; (III) spatial transport through layered PVA and diaphragm–cell geometries; (IV) thermal and antibiotic effects on survival and culturability; and (V) recolonization and delayed bacterial recovery. Experimental inputs included beaker exchange trials, continuous-flow dispersion, spatially phantom PVA trials, diaphragm–cell transport, localized heating, and antibiotic exposure.

**Results:** Approximately 90% of the initial recoverable surface population disappeared from the biofilm phase within the first measured 30 s of liquid exposure, while subsequent fresh-media rinses released much smaller populations. Flow-cell experiments showed a strong initial bacterial output followed by sustained lower release. In PVA, viable bacteria were recovered millimeters from the biofilm at ambient temperature and throughout layers extending to approximately 1.2 cm at physiological temperature. Thermal treatments (50 – 80 °C) increased viable penetration distance of bacteria under study, whereas sufficiently strong treatment (80 °C for 3 minutes) eliminated detectable surface and PVA bacterial populations. Additionally, bacteria retained in the surrounding PVA could establish a new biofilm on a sterile replacement coupon within 24 h.

**Conclusion:** The framework indicates that biofilm treatment should be evaluated as a spatial population-control problem rather than by surface log reduction alone. Rapid release can create a surviving external reservoir, and durable bacterial control depends on both reducing the treated surface population and limiting the ability of dispersed bacteria to survive, and recolonize. The model provides a structure for designing experiments that separately identify these coupled processes and ultimately predict whole-domain treatment outcomes.

## Poster #10

**Ramkrishna Sen**

Actively targeting colorectal cancer using nanoparticles co-loaded with small molecule inhibitors

**Background:** The combination of BRAF V600E inhibitors (e.g., encorafenib, dabrafenib) and MEK inhibitors (e.g., trametinib, binimetinib) offers second-line therapy for metastatic colorectal cancer (CRC) with BRAF V600E mutations, effectively blocking the MAPK/ERK pathway. However, poor solubility, bioavailability, nonspecific distribution, and resistance limit its potential as a primary therapy. To address this, we developed cRGD-functionalized nanoparticles co-loaded with encorafenib and trametinib (cRGD-ET-NPs), enhancing efficacy at maximum tolerated doses in a murine xenograft CRC model.

**Objectives:**

1. Formulation and Characterization: Develop cRGD-functionalized nanoparticles co-loaded with encorafenib and trametinib (cRGD-ET-NPs) and characterize their physicochemical properties, drug loading, and release profiles.
2. In Vitro Evaluation and Tumor Targeting: Evaluate cellular uptake and penetration of targeted versus nontargeted nanoparticles in HT-29 cells and 3D tumor spheroids, and investigate in vivo tumor localization and biodistribution using IVIS imaging.
3. In Vivo Antitumor Efficacy: Compare the therapeutic efficacy of cRGD-ET-NPs with nontargeted ET-NPs and free encorafenib–trametinib combination therapy in a BRAF V600E-mutated colorectal cancer xenograft mouse model.

**Methods:** We developed cRGD-ET-NPs using a nanoprecipitation method [1] for the treatment of CRC. Both the targeted (cRGD-ET-NPs) and nontargeted (ET-NPs) formulations were characterized by DLS, TEM, and XPS. Anticancer activity was assessed in HT-29 cells; cellular penetration study was analyzed using 3D tumor spheroids. We performed IVIS imaging to determine in vivo tumor localization and biodistribution in an animal model. In a murine xenograft model, HT-29 cells were injected into nude mice. Once tumors reached  $\sim 200 \text{ mm}^3$ , mice were treated with DPBS (control), free ET (oral), ET-NPs (IV) and cRGD-ET-NPs (IV). Treatments were administered on days 0, 3, 7, and 11, with oral doses of encorafenib (60 mg/kg) and trametinib (0.6 mg/kg), while ET-NPs and cRGD-ET-NPs were delivered IV.

**Results:** The average hydrodynamic diameter (dH) of ET-NP and cRGD-ET-NPs was found to be  $40.16 \pm 2.53 \text{ nm}$  and  $46.31 \pm 1.22 \text{ nm}$ , respectively. The drug loading of ET-NPs was determined to be  $86.40 \pm 17.47 \text{ } \mu\text{g}/\text{mg}$  for encorafenib and  $0.68 \pm 0.08 \text{ } \mu\text{g}/\text{mg}$  for trametinib. Similarly, cRGD-ET-NPs exhibited a drug loading of  $69.17 \pm 15.63 \text{ } \mu\text{g}/\text{mg}$  for encorafenib and  $0.58 \pm 0.06 \text{ } \mu\text{g}/\text{mg}$  for trametinib. Surface conjugation of cRGD to ET-NPs was confirmed by XPS analysis. The drug release of ET from the NPs followed a biphasic pattern, showing a characteristic burst release within the initial 24 h, followed by a slow, sustained release over the next 168 h. Three-D spheroid models confirmed superior intratumoral penetration of fluorescently labeled cRGD-NPs over fluorescently labeled nontargeted NPs within the three-dimensional space; and this was reflected in the improved the overall antitumor efficacy of cRGD-ET-NPs over ET-NPs in vivo. Enhanced in vivo tumor localization and biodistribution of targeted versus nontargeted DiR-loaded NPs in vivo was confirmed by IVIS imaging.

**Conclusion:** Using a CRC (BRAfV600E) xenograft mouse model, it was shown that the combination of the BRAF inhibitor, encorafenib, and the MEK inhibitor, trametinib, showed significant anti-tumor activity when co-loaded into targeting NPs compared to the soluble combination. In vitro data indicated that this enhanced tumor activity was likely due to enhanced uptake and penetration. This study establishes co-delivery of SMIs using nanoformulations as superior to delivery in soluble form and therefore may provide enhanced therapy when implemented clinical settings.

## Poster #11

Lilly Duff

### Single molecule mechanics reveals convergent allostery in glutamate racemase

**Background:** *Helicobacter pylori* is a major cause of gastric cancer, while antibiotic resistance increasingly complicates treatment. Current multidrug therapies can also disrupt the healthy gut microbiome, motivating the development of species-selective antibiotics. Glutamate racemase (Muri), an essential enzyme in bacterial cell-wall biosynthesis, is a promising target for selective inhibition. Previous work identified allosteric inhibitors that bind the *H. pylori* Muri (HpMuri) dimer interface and demonstrated that iterative optimization of these compounds can improve antibacterial activity. However, developing potent enzyme inhibitors that also effectively inhibit bacterial growth remains a challenge.

**Objectives:** This project aims to advance novel HpMuri inhibitors through experimental characterization and iterative structure-guided optimization. We seek to identify compounds with potent enzyme inhibition and antibacterial activity, establish structure-activity relationships (SAR) that guide chemical modifications, and prioritize promising candidates for further development.

**Methods:** Building on a previously validated molecular docking workflow, candidate compounds targeting the HpMuri dimer interface are evaluated using a coupled enzymatic activity assay to quantify inhibition of HpMuri-catalyzed glutamate racemization. Compounds demonstrating promising activity are further evaluated against *H. pylori* using minimum inhibitory concentration (MIC) assays to assess antibacterial efficacy. Enzyme inhibition and MIC results are integrated with structural analyses to identify relationships between chemical features, target inhibition, and bacterial growth suppression. These findings guide the selection and design of derivatives for subsequent rounds of synthesis and testing.

**Results:** Previous work established a structure-guided screening strategy for identifying HpMuri inhibitors and demonstrated that iterative chemical optimization can improve both enzyme inhibition and antibacterial activity. Notably, structural modifications produced substantial differences in enzyme potency and bacterial growth inhibition, highlighting the importance of evaluating both properties during lead optimization. The current study extends this approach through experimental characterization of 32 additional candidate compounds, with ongoing investigations aimed at identifying promising chemical scaffolds and defining their structure-activity relationships.

**Conclusion:** This research advances a systematic drug discovery strategy for developing HpMuri inhibitors by integrating computational screening, biochemical characterization, antibacterial testing, and iterative medicinal chemistry. By identifying compounds that combine potent enzyme inhibition with effective suppression of *H. pylori* growth, this work aims to establish promising leads for species-selective antibiotic development. Ultimately, this approach could contribute to more targeted treatments for *H. pylori* infection while informing broader strategies for discovering antibiotics against Gram-negative pathogens.

## Poster #12

**Rose Gogal**

The prevalence of protein misfolding as a mechanism for hereditary deafness

**Background:** The Deafness Variation Database (DVD) is a comprehensive, open-access resource from the Molecular Otolaryngology & Renal Research Laboratories (MORL) at the University of Iowa that collects, annotates, and classifies genetic variants associated with hearing loss. It contains ~380,000 missense variants across 224 genes with over 300,000 (~75%) having an unknown significance (VUS).

**Objectives:** With the emergence of deep learning methods for protein structure prediction, we sought to combine genetic information with biophysical evidence to improve the classification of these variants.

**Methods:** We applied AlphaFold3 to predict protein structures for all genes and their corresponding isoforms in the DVD. We then optimized these structures with the AMOEBA polarizable force field to resolve steric clashes, repack sidechains and improve backbone conformations. The optimized protein structures were used as input to the folding free energy difference ( $\Delta\Delta G_{\text{Fold}}$ ) prediction tool DDGun; when high, positive  $\Delta\Delta G_{\text{Fold}}$  indicates a significant disruption in folding.

**Results:** Using guidelines set in Pejaver et al. for determining the strength of computational evidence from tools like Revel, we constructed a likelihood function that combines  $\Delta\Delta G_{\text{Fold}}$  and Revel, and used Bayesian analysis to identify those VUSs very likely to cause hearing loss. The Bayesian formulation established by combining  $\Delta\Delta G_{\text{Fold}}$  with Revel identified ~20,000 missense VUSs with very strong evidence of being deleterious at a false positive rate of <0.1%.

**Conclusion:** Using deep learning protein structure prediction models, sequence-based genetic predictors, and biophysical evidence, we identified missense variants highly likely to lead to protein misfolding. Future work will link these data to patients who carry prioritized missense variants but did not receive a definitive genetic diagnosis for their hearing loss to determine whether variant classification can be upgraded from VUS to likely pathogenic.

## Poster #13

**Anna Claire**

Muscle phosphofructokinase as a potential link between mechanics, metabolism and gene expression

**Background:** Cells are subjected to mechanical forces, which are detected by cell adhesion receptors that trigger actin cytoskeletal remodeling, adhesion complex growth to counteract these forces, and expression of genes that induce proliferation. Previously, the laboratory discovered that the muscle isoform of phosphofructokinase-1 (PFKM), the rate-limiting and committed enzyme of glycolysis, provides the cell with the required energy to respond to these forces. Inhibiting PFKM disrupts glucose uptake, actin cytoskeletal reinforcement, and cell-cell adhesion reinforcement, thereby suggesting that PFKM links mechanics and metabolism. How these events are coordinated with gene expression however remains poorly understood. Clues for how this might occur come from a recent study suggesting that PFKM induces gene expression by binding to and phosphorylating histone H3 in response to high levels of citrate.

**Objectives:** I aim to test the hypothesis that shear stress activates PFKM-mediated phosphorylation of histone H3 which stimulates cell proliferation.

**Methods:** So far, I have employed shear stress to parental MCF10A, PFKM knockdown, PFKM addback, and histone H3 knockdown cells and immunoblotted for histone H3 S10 phosphorylation. Moving forward I hope to employ similar techniques with phosphomimetic and phospho-deficient histone H3 mutants and kinase dead PFKM mutant as well as RNAseq and proliferation assays with all cell lines.

**Results:** Shear Stress stimulates phosphorylation of histone H3 at S10 in a PFKM dependent manner.

**Conclusion:** The expected outcome of this study is that it will establish PFKM-mediated phosphorylation of histone H3 as a key determinant of cell fortification and proliferation in response to force. In doing so, these studies establish the first connections between three seemingly disparate processes: mechanics, metabolism, and gene expression. This information is critical for the identification of targets for the treatment of proliferative diseases, such as cancer, that arise from metabolic and mechanical defects.

## Poster #14

**Luke Handlos**

### Oligomeric States and Conformational Flexibility of the DNA Damage Bypass Complex Rad6–Rad18

**Background:** DNA lesions from oxidative stress, ultraviolet (UV) radiation, and chemical mutagens can stall DNA polymerases (e.g., pol  $\delta$ ,  $\epsilon$ ), threatening genomic instability. Cells avoid this by employing Rad6–Rad18 to monoubiquitylate PCNA, promoting translesion synthesis (TLS), which recruits specialized TLS polymerases to bypass DNA lesions. Recent evidence suggests that oligomeric states of Rad6–Rad18 influence TLS polymerase recruitment: higher-order species interact with pol  $\eta$  recruitment, whereas Rad18 monomers or dimers interact with Rev1. Because both TLS polymerases differ in mutagenic outcomes, Rad6–Rad18 may act as a master regulator for TLS polymerase recruitment that determines an error-prone or error-free outcome. However, the oligomeric state(s) and conformational flexibility of Rad6–Rad18 remain poorly characterized.

**Objectives:** We sought to characterize the oligomeric states, domain arrangements, and conformational flexibility of Rad6–Rad18 in solution and identify the species that best explain small-angle X-ray scattering (SAXS) data.

**Methods:** Evolving factor analysis (EFA) was performed on Rad6–Rad18 SAXS data to identify distinct scattering components characterized by their radius of gyration ( $R_g$ ) and maximum particle dimension ( $D_{max}$ ). Candidate oligomeric states of Rad6–Rad18 were generated using AlphaFold3 and subjected to triplicate 1- $\mu$ s Langevin dynamics simulations. Structural snapshots from simulations were compiled into ensembles, and theoretical scattering curves were generated and compared to the scattering components using  $\chi^2$  analysis. Ensembles were further re-weighted in linear combinations to identify oligomeric species mixtures that best fit each scattering component.

**Results:** EFA identified two scattering components with  $R_g/D_{max}$  values of 28/90 Å and 41/150 Å. Most simulated Rad6–Rad18 species maintained compact conformations, whereas the Rad18 monomer and dimer sampled a broader conformational space. Direct comparisons of individual ensembles identified Rad6 and the Rad6<sub>1</sub>–Rad18<sub>1</sub> as the best-fitting species for components 1 and 2, respectively, with  $\chi^2$  values of 1.55 and 1.42. Mixture analysis further identified the combination of 67% Rad6 alone and 33% Rad6<sub>1</sub>–Rad18<sub>1</sub> as the best fit for component 1 ( $\chi^2 = 1.08$ ), while component 2 was best described by 83% Rad6<sub>1</sub>–Rad18<sub>1</sub> and 17% Rad6<sub>1</sub>–Rad18<sub>2</sub> (trans) ( $\chi^2 = 1.40$ ). Pairwise comparisons revealed alternative mixtures with similarly low  $\chi^2$  values, with component 1 having a Rad6/Rad18 mixture fit comparably to the Rad6/Rad6<sub>1</sub>–Rad18<sub>1</sub>, with a  $\chi^2$  value of 1.14. For component 2, mixtures of Rad6<sub>1</sub>–Rad18<sub>1</sub> with cis or trans Rad6<sub>2</sub>–Rad18<sub>2</sub> fit nearly as well as the Rad6<sub>1</sub>–Rad18<sub>1</sub>/Rad6<sub>1</sub>–Rad18<sub>2</sub> (trans) mixture, with  $\chi^2$  values of 1.41, leaving confidence in component 2's precise composition less certain than component 1.

**Conclusion:** From these results, we propose a sequential assembly model positing that Rad6 and Rad18 can rapidly interconvert with the Rad6<sub>1</sub>–Rad18<sub>1</sub> complex. Similarly, the Rad6<sub>1</sub>–Rad18<sub>1</sub> complex can rapidly interconvert with the Rad6<sub>1</sub>–Rad18<sub>2</sub> (trans) complex and possibly higher-order species such as Rad6<sub>2</sub>–Rad18<sub>2</sub>. Given that we identified these regimes in two distinct scattering components, this suggests a slow exchange between them. This potentially reflects a rate-limiting step such as a structural rearrangement of the Rad6<sub>1</sub>–Rad18<sub>1</sub> complex for a second Rad18 protomer to bind to form the Rad6<sub>1</sub>–Rad18<sub>2</sub> (trans) complex. Overall, this work provides insight into the conformational flexibility and potential species involved in Rad6–Rad18 complex assembly.

## Poster #15

**Edozie Samuel  
Okpara**

Investigating the Cell-Type-Specific Effects of miR29a-KLF4 and miR29a-ADAM12 signaling in Peripheral Arterial Disease (PAD)

**Background:** Peripheral arterial disease (PAD) causes severe disability and limb loss, particularly in diabetes. Because impaired muscle regeneration and vascular recovery drive PAD progression, and microRNA-29a (miR-29a) regulates ischemic repair by targeting angiogenesis and remodeling genes (ADAM12, KLF4), this study investigates the cell-type-specific effects of ischemia on miR-29a, KLF4, and ADAM12 expression in endothelial cells versus myoblasts.

**Objectives:** To investigate the cell-type-specific effects of ischemia on miR-29a, KLF4, and ADAM12 expression in skeletal muscle microvascular endothelial cells (ECs) and myoblasts (MBs) .

**Methods:** Mouse skeletal muscle microvascular endothelial cells and myoblasts were exposed to simulated ischemia (2% O<sub>2</sub> with nutrient deprivation). Total RNA was isolated, and miR-29a, KLF4, and ADAM12 expression were quantified using TaqMan and SYBR Green qPCR and compared with control group.

**Results:** Ischemia significantly altered target expression in endothelial cells, decreasing miR-29a and ADAM12 while increasing KLF4 expression. Conversely, myoblasts exhibited minimal or no significant changes. Direct comparison demonstrated a substantially greater ischemic response in endothelial cells than in myoblasts, revealing distinct cell-specific regulation of the miR-29a-KLF4 and miR-29a-ADAM12 axes.

**Conclusion:** Although ischemia induces distinct, cell-dependent changes in miR-29a, KLF4, and ADAM12 expression within endothelial cells—supporting the therapeutic potential of targeting endothelial miR-29a for vascular recovery—the lack of similar changes in myoblasts suggests that the effects of ischemia should be further tested in mature myofibers.

## Poster #16

**JoJo Peplinski**

Investigating Stromal-Modulating Agents to Alter Tumor Mechanics and Enhance Drug Delivery in Pancreatic Ductal Adenocarcinoma

**Background:** Pancreatic ductal adenocarcinoma (PDAC) is characterized by a dense, desmoplastic stroma that contributes to altered tumor mechanics and can limit therapeutic delivery. Stromal-modulating agents may alter extracellular matrix (ECM) composition and tumor mechanical properties, potentially improving intratumoral drug transport. However, how stromal-modulating treatments alter PDAC tumor mechanics in relation to ECM composition remains incompletely understood.

**Objectives:** This study aims to determine whether stromal-modulating agents alter the mechanical properties of PDAC tumors and whether these changes are associated with ECM composition and in vivo tumor stiffness measured by shear wave elastography (SWE). Ex vivo rheological properties, including storage modulus ( $G'$ ), stress relaxation behavior, and compressive properties, are evaluated. Ultimately, this work seeks to determine whether tumor mechanical properties are associated with intratumoral drug penetration.

**Methods:** Subcutaneous PDAC tumors were established in mice using KPC-luc cells, and mice received daily treatment with candidate stromal-modulating agents, including losartan. SWE was performed when tumors reached approximately 1,000–1,400 mm<sup>3</sup>, followed by tumor harvest at approximately 1,500 mm<sup>3</sup>. Tumors were sampled using 8-mm biopsy punches and analyzed using a TA Instruments DHR-1 rheometer. Strain sweeps characterized storage modulus ( $G'$ ), stress relaxation testing assessed time-dependent mechanical behavior, and axial compression assessed compressive properties. The maximum modulus value reported by the rheometer was divided by the measured gap to obtain a gap-normalized value. Masson's trichrome staining was used for preliminary histological assessment of collagen, with additional analyses planned to characterize ECM composition and stromal markers.

**Results:** Losartan-treated tumors showed trends toward decreased storage modulus ( $G'$ ), compressive Young's modulus ( $E$ ), and in vivo shear modulus ( $G$ ) measured by shear wave elastography (SWE); however, none of these differences reached statistical significance. Stress relaxation behavior showed little apparent change following treatment. Preliminary visual assessment of Masson's trichrome staining suggested increased blue staining in losartan-treated tumors compared with controls. Further quantitative analysis is needed to determine whether this observation reflects differences in collagen content.

**Conclusion:** Preliminary findings suggest that losartan treatment may be associated with reductions in PDAC tumor mechanical properties, with directional trends observed across ex vivo rheological measurements and in vivo shear wave elastography. Although these changes were not statistically significant, further investigation is warranted. Future work will quantify ECM composition, evaluate additional stromal-modulating agents, and investigate whether treatment-associated changes in tumor mechanics correlate with intratumoral drug penetration.

## Poster #17

**Rachel Kehrberg**

Fatty Acid Oxidation Restrains Mechanically Induced Junctional Reinforcement Through Effects on Membrane Lipid Composition

**Background:** All cells experience mechanical forces. To withstand these forces, cells must reinforce their actin cytoskeleton and cell-cell junctions through energy intensive processes, including protein synthesis, actin polymerization, and gene expression. How cells meet the energetic demands of these adaptations is an area of intense scrutiny. We and others have previously demonstrated a role for glycolysis, but the role of lipid metabolism is less well understood. The rate limiting step of fatty acid oxidization occurs via carnitine palmitoyltransferase 1a (Cpt1a), which catalyzes the conjugation of carnitine to long chain acyl CoAs to enable their import into mitochondria.

**Objectives:** To determine how fatty acid metabolism contributes to the epithelial response to mechanical force and to define the role of Cpt1a in coordinating metabolic and structural responses to shear stress.

**Methods:** NMuMG, MCF10A, and MDCKII epithelial cells were exposed to 12 dynes/cm<sup>2</sup> orbital shear stress. Metabolic changes were assessed by mass spectrometry. Cpt1a knockdown cell lines were generated using shRNA. Junctional actin and  $\beta$ -catenin were quantified by immunofluorescence microscopy.

**Results:** Shear stress increased cellular and mitochondrial acylcarnitine levels, indicating activation of Cpt1a-dependent fatty acid metabolism. These force-stimulated elevations occurred through a signal transduction pathway from E-cadherin to AMPK-mediated activation of the ACC-malonyl-CoA signaling pathway. Surprisingly, genetic or pharmacologic inhibition of Cpt1a enhanced shear stress-induced junctional reinforcement. This phenotype was rescued by re-expression of wild-type Cpt1a, but not a catalytically inactive G710E mutant, demonstrating a requirement for Cpt1a enzymatic activity. Metabolomic analysis revealed that loss of Cpt1a redirected acyl-CoAs away from fatty acid oxidation and toward phospholipid remodeling. Cpt1a-deficient cells exhibited increased expression of membrane-bound O-acyltransferase domain-containing 2 (Mboat2), accumulation of 18:1-containing phospholipids, and reduced membrane fluidity. Inhibition of mammalian target of rapamycin (mTor), a signaling pathway sensitive to membrane biophysical properties, reversed the enhanced reinforcement observed following Cpt1a loss.

**Conclusion:** In response to shear stress, cells increased Cpt1a-mediated fatty acid oxidation. Loss of Cpt1a led to phospholipid remodeling, decreased membrane fluidity, and increased junctional reinforcement via mTor.

## Poster #18

**Atonu Chakraborty**

### Temporal Remodeling of ABC Transporter Programs During Acquired Paclitaxel Resistance in Ovarian Cancer

**Background:** Ovarian cancer remains difficult to treat because many patients eventually develop resistance to chemotherapy. Paclitaxel is widely used in ovarian cancer treatment, but its effectiveness often decreases as cancer cells become resistant. My research focuses on ATP-binding cassette (ABC) transporters, proteins that can move drugs and other molecules across cell membranes. Although some transporters are known to contribute to drug resistance, attempts to overcome resistance by targeting individual transporters have largely been unsuccessful. This makes me interested in understanding how multiple transporters change during treatment and whether they contribute to resistance together.

**Objectives:** My main objective is to understand how ovarian cancer cells adapt to paclitaxel and what allows them to remain resistant. I am particularly interested in identifying which ABC transporters are functionally important during different stages of resistance. One focus is ABCC3, which is increased in our resistant ovarian cancer model. I also want to investigate whether developing chemotherapy resistance changes how cancer cells are recognized by immune cells, particularly Vγ9Vδ2 T cells.

**Methods:** We developed paclitaxel-resistant ovarian cancer cell models through repeated drug exposure and recovery. I use RNA sequencing to compare gene expression in sensitive cells, cells exposed to paclitaxel for 6 or 48 hours, and established resistant cells. To investigate the function of specific transporters, I use gene knockdown, drug sensitivity assays, and measurements of cell growth and apoptosis. I also plan to use fluorescence microscopy and flow cytometry to examine how ABCC3 affects paclitaxel distribution inside cells. For the immune component, I will use co-culture experiments to study interactions between ovarian cancer cells and Vγ9Vδ2 T cells.

**Results:** Our RNA-seq results show that ABC transporter expression changes considerably as ovarian cancer cells develop paclitaxel resistance. Interestingly, these changes differ between early drug exposure and established resistance, as well as between different ovarian cancer cell models. In OV90 cells, ABCC3 expression increases substantially after acquired resistance develops, making it a potential contributor to maintaining resistance. We also observed reduced BTN3A1 expression in resistant cells. Since BTN3A1 is involved in Vγ9Vδ2 T-cell recognition, this finding raises questions about whether chemotherapy resistance also affects immune recognition. We are now testing whether these changes have direct functional consequences.

**Conclusion:** Our findings suggest that paclitaxel resistance involves changes in multiple transporters rather than a single protein. This may partly explain why previous approaches targeting individual transporters have been unsuccessful. By investigating the functional roles of ABCC3 and BTN3A1, I hope to understand both how ovarian cancer cells maintain resistance and whether resistance affects their interactions with immune cells. Ultimately, identifying these mechanisms could help us find new ways to restore chemotherapy sensitivity and improve treatment outcomes for patients with resistant ovarian cancer.

## Poster #19

**Joseph Correa**

### Rapidly Dissolving Microneedles for Acute Anaphylaxis

**Background:** Epinephrine is the first-line treatment for acute anaphylaxis. Typically delivered with autoinjectors, it has a rapid onset of action and reaches peak efficacy within ten minutes. However, with a half-life less than five minutes, efficacy can be short-lived, which often requires multiple doses before medical care arrives. Transdermal drug delivery systems offer painless administration, customizable release profiles, and patient favorability over injections.

**Objectives:** To limit the need for additional doses while still providing fast relief, we are developing polymeric dissolving microneedles (dMNs) for painless and rapid epinephrine delivery with continued release.

**Methods:** In vitro drug release studies were conducted using static Franz diffusion cells to assess the epinephrine release profile from gel formulations with polymer bases including pullulan (PL), polyvinyl pyrrolidone (PVP), hyaluronic acid (HA), and hydroxyethyl cellulose (HEC). The gels were cast into dMN arrays via a mold centrifugation method, and the arrays were subjected to axial compression to evaluate the mechanical strength of the arrays. Sufficiently strong arrays were investigated with ex vivo permeation studies across excised porcine skin. Dissolution studies were performed with dMNs made of PL, PVP, hydroxypropyl methylcellulose (HPMC), and polyvinyl alcohol (PVA) in which the arrays were inserted into excised porcine skin, removed at predetermined times, and imaged with a microscope to evaluate the extent of dissolution.

**Results:** We screened polymers based on epinephrine release, dMN forming ability, and epinephrine permeation across porcine skin. Of the polymers investigated, PL and PVP had the highest epinephrine release, releasing up to 63% of their loading in 30 minutes. PL dMNs showed superior mechanical strength, withstanding 20 N of force without breaking. In permeation studies, 10% (w/w) PL with 2% (w/w) epinephrine demonstrated that dMNs improved permeation versus intact skin controls. However, quantifiable epinephrine concentrations were not detected until 30 minutes, which may be attributed to the dMN dissolution rate. To overcome this limitation, we are optimizing the dMN formulation to achieve >90% dissolution within 60 seconds, thus allowing earlier epinephrine permeation. 15% and 30% (w/w) PVA formed strong dMN arrays that withstood breaking upon insertion but demonstrated no dissolution within 60 seconds. 2% (w/w) HPMC required a 10% (w/w) PL backing layer to produce well-formed arrays. However, the HPMC needles could not pierce the skin. 30% (w/w) PVP arrays withstood breaking upon insertion and achieved >80% dissolution within 30 seconds. The mechanical properties of the arrays depended heavily on preparation techniques, with small adjustments, such as when to add the backing layer, correlating with large changes in mechanical strength.

**Conclusion:** Epinephrine can be delivered through the skin utilizing dMNs, but fast delivery requires rapid dissolution of the MNs. PVP can release epinephrine rapidly, and dMNs made of PVP demonstrated near complete dissolution in 30 seconds, which is promising for the next steps in developing a transdermal epinephrine delivery system. Future studies will investigate the correlation of dMN dissolution rate and epinephrine permeation and will evaluate the effect of localized warming on epinephrine permeation.

## Poster #20

**Andrew Thiel**

Polarizable Constant pH Molecular Dynamics for Protein-Cofactor and Protein-DNA Complexes

**Background:** Constant pH molecular dynamics (CpHMD) algorithms are valuable for investigating biological mechanisms in which molecular conformation and protonation are coupled. CpHMD methods have been limited to fixed-charge representations of electrostatics, impeding their capability to investigate highly polar environments such as those surrounding DNA-binding domains.

**Objectives:** To overcome the limitation of previous model, the first CpHMD algorithm for the Atomic Multipole Optimized Energetics for Biomolecular Applications (AMOEBA) force field was developed.. Here, we apply AMOEBA CpHMD to attain CpHMD pKa predictions to date for three well-studied proteins. We also apply AMOEBA CpHMD simulations to the highly polarizable cysteine-bound Zn<sup>2+</sup> ion and protein-DNA complexes. Fixed-charge models struggle to accurately predict cysteine pKa shifts and we will show that inclusion of polarizability improves upon those predictions.

**Methods:** AMOEBA CpHMD simulations are run on Hen Egg White Lysozyme (HEWL), the Chicken Villin Headpiece Subdomain (HP36), and BBL. We also perform CpHMD simulations of Zinc fingers in which four cysteines are involved in binding the Zn<sup>2+</sup> ion, which is a highly conserved motif in transcription factors. We investigate titration state changes in the absence and presence of DNA. Lastly, we assess the impact of cysteine protonation states changes on DNA binding with free energy calculations.

**Results:** The root-mean-square-deviation from experiment was 0.59 with a Pearson's correlation coefficient of 0.90. This marks an improvement over the fixed-charge methods, which is highlighted by significant improvement for the prediction of the catalytic dyad in HEWL (GLU-35/ASP-52) with near agreement to experiment. The fixed-charge models were shown to overestimate the ASP-52 pKa by as much as 2 pH units.

**Conclusion:** Validation of polarizable CpHMD on three well-studied proteins demonstrated the most accurate predictions of pKa to date over previous models and may be used to improve our understanding of transcription factor binding affinity/specificity.

## Poster #21

**Pornpoj  
Phruttivanichakun**

Growth Differentiation Factor 5 (GDF5) as a potential therapy for post-traumatic osteoarthritis.

**Background:** Post-traumatic osteoarthritis (PTOA) is a progressive degeneration of the joint cartilage that is accompanied by pain, mobility difficulties, and eventually the need for joint surgery. So far, there is no pharmacological treatment that can effectively promote cartilage healing to prevent PTOA progression and to regenerate damaged cartilage. Cartilage progenitor cells (CPCs) are a group of cells known to migrate to damaged regions and support repair processes. We hypothesized that pharmacologically activating and/or recruiting CPCs to damaged cartilage would promote healing and cartilage regeneration in PTOA.

**Objectives:** In this study, we evaluated the in vitro and ex vivo effects of Growth Differentiation Factor 5 (GDF5), an endogenous growth factor, on CPC migration and chondrogenic differentiation in the presence and/or absence of a pharmacologically induced inflammatory condition.

**Methods:** Bovine CPCs were isolated from the surface of stifle joint cartilage explants, following an ex vivo mechanical injury, and cultured per our previous study[PP1.1]. CPC migration following recombinant GDF5 treatments was quantified using a transwell migration assay, while CPC proliferation after GDF5 treatments was determined by measuring total dsDNA contents. The chondrogenic properties of GDF5 in CPCs were evaluated in cell monolayers and in cell pellets using different assays as follows: real-time quantitative PCR (RT-qPCR) for chondrogenic markers, glycosaminoglycans (GAGs) quantification using DMMB assay, pellet wet weight monitoring, and Safranin-O/fast green histological staining. The anti-catabolic effect of GDF5 was determined by assessing the downregulation of ADAMTS5 (RT-qPCR), a key aggrecanase involved in the articular matrix degradation, under either normal or interleukin 1 $\beta$ -induced inflammatory conditions. Bulk RNA sequencing was also performed to examine the effects of GDF5 treatment in CPCs on gene expressions related to glycolysis.

**Results:** GDF5 demonstrated a robust chemoattractant effect on CPCs, evidenced by a significantly higher number of migrated CPCs in GDF5-treated groups compared to untreated controls, with migration increasing in a dose-dependent manner. GDF5 treatment significantly promoted CPCs proliferation by promoting glycolytic metabolism. In CPC monolayers, GDF5 significantly upregulated chondrogenic markers (SRY-box transcription factor 9, SOX9; collagen type II alpha 1, COL2a1; and aggrecan, ACAN) in a dose-dependent manner. On the other hand, in CPC pellets, GDF5 induced chondrogenic differentiation as indicated by increases in pellet wet weights, enhanced GAGs production, and heightened proteoglycan content. Furthermore, GDF5 significantly reduced the expression of ADAMTS5 in CPCs under both normal and inflammatory conditions.

**Conclusion:** We demonstrated the potential of GDF5 as a therapeutic protein in CPC-based cartilage regeneration. In summary, GDF5 significantly promoted CPC migration, proliferation, and chondrogenic differentiation in both cell monolayer and cell pellet models. GDF5 also significantly downregulated ADAMTS5, indicating the GDF5 role in preventing cartilage matrix degradation. These findings position GDF5 as a promising candidate for promoting CPC-based cartilage regeneration and warrants its further investigation as an effective therapy to prevent PTOA progression.

## Poster #22

**Jake Miller**

Accurate Prediction of Protein-DNA Relative Binding Free Energies Using the AMOEBA Force Field

**Background:** Protein-DNA recognition underlies genome replication, DNA damage response, and transcriptional regulation. Genetic variants that disrupt these interactions can dysregulate transcription programs and contribute to disease. Experimental characterization of variant effects is costly and time-consuming, motivating the use of computational methods to estimate changes in binding free energy ( $\Delta\Delta G_{\text{Bind}}$ ) due to a variant in the protein or the DNA. There have been few published methods to calculate  $\Delta\Delta G_{\text{Bind}}$  for complementary base pair DNA variants, and all have used molecular dynamics with fixed-charge force fields. Protein-DNA interfaces are governed by strong, environment-dependent electrostatics that fixed-charge force fields, which assign static atomic point charges, cannot fully capture. The polarizable AMOEBA (Atomic Multipole Optimized Energetics for Biomolecular Applications) force field addresses this by representing permanent electrostatics with atomic multipoles and explicitly modeling polarization through induced dipoles, but a practical relative binding free energy methodology with AMOEBA for protein-nucleic acid systems has not previously existed.

**Objectives:** The goal of this work is to establish and validate a reproducible AMOEBA-based relative binding free energy difference methodology across a benchmark set of protein-DNA systems with known experimental binding affinities, directly comparing AMOEBA results to fixed-charge force field calculations to quantify the accuracy gained from explicitly modeling polarization.

**Methods:** We developed the first GPU-accelerated dual-topology AMOEBA implementation by modifying OpenMM and controlling simulations with Force Field X, which enabled calculation of  $\Delta\Delta G_{\text{Bind}}$  for a wild-type/variant base pair within a single simulation rather than two independent absolute binding free energy calculations. A global parameter,  $\lambda$ , scales the nonbonded (van der Waals, permanent electrostatics, polarization) terms between end states. Equilibrium molecular dynamics at fixed  $\lambda$  values were used to sample the transition between the end states, and free energy differences are estimated with the Bennett Acceptance Ratio (BAR). We validated the methodology on three protein-DNA systems with diverse binding motifs and sizes – the AterF1 transcription factor, the EcoRI endonuclease, and the MAT  $\alpha 1$ - $\alpha 2$  transcription repressor heterodimer – each with nine base-pair variants (29 variants total), benchmarking against experimental affinities and published fixed-charge force field (Amber99sb\*-ILDN-BSC1, CHARMM36m) results for the same systems.

**Results:** Across all 29 variants, AMOEBA achieved a mean unsigned error (MUE) of 1.16 kcal/mol and a Pearson correlation of 0.79 with experiment, approaching our chemical accuracy target of ~1.0 kcal/mol and substantially outperforming the fixed-charge force fields (Amber: MUE = 2.40 kcal/mol,  $r = 0.26$ ; CHARMM: MUE = 2.06 kcal/mol,  $r = 0.30$ ). Accuracy varied modestly by variant type: purine-to-purine substitutions, which involve fewer alchemical atoms, converged more readily (MUE = 0.98 kcal/mol) than purine-to-pyrimidine substitutions (MUE = 1.28–1.31 kcal/mol).

**Conclusion:** These results support our central hypothesis that explicitly representing electronic polarization and higher-order electrostatics improves the accuracy of relative binding free energy predictions for protein-DNA interactions. The validated dual-topology AMOEBA relative binding free energy difference framework provides a reproducible, open-source methodology for probing how DNA sequence variation alters protein binding. In the future, we aim to improve our sampling methods to accelerate  $\Delta\Delta G_{\text{Bind}}$  convergence and overcome energy barriers, and to apply the framework to disease-associated genetic variants.

## Poster #23

**Sarah Torrence**

Recombinant Prohibitins Mitigate Pro-Inflammatory Priming Response and Acute Kidney Injury Induced by Lipopolysaccharide

**Background:** Prohibitins 1 and 2 (PHB1/2) are ubiquitously expressed lipid raft proteins that form a multimeric ring complex in membranes where they regulate numerous cell processes including growth, signaling, gene activation/transcription, and metabolism. PHBs are interdependent, such that knockout of one results in degradation of the other, yet also have distinct biological effects independent of each other. Work in our lab and published literature consistently supports a cyto-protective and anti-inflammatory role for these proteins. Previous research in our lab demonstrated that recombinant PHB1 (rPHB1) has anti-inflammatory and cardioprotective effects in lipopolysaccharide (LPS)-induced endotoxemia in mice, and that this effect is PI3K-dependent.

**Objectives:** In this study we sought to determine whether rPHB1 also protects mice from acute kidney injury (AKI) induced by LPS, and to further investigate the protective effects of rPHB's in HK-2 cells, a proximal tubular cell line derived from human kidney.

**Methods:** Adult male C57/Bl6J mice were randomly treated with an intraperitoneal injection of saline (vehicle), LPS (12 mg/kg b.w.), rPHB1 (300 ng), and LPS + rPHB1 combined, and euthanized 18 hours later. Kidney tissue from the mice were evaluated via standard histological technique for tubular swelling and injury. We used a variety of commercial fluorescence-based assays to probe metabolic activity and cytotoxicity in HK-2 cells treated with LPS and rPHB1 and rPHB1. We also performed confocal microscopy to determine cellular localization of recombinant PHB1.

**Results:** The results of these assays show that treatment with LPS and ATP induces an inflammatory state in HK-2 cells marked by increased ROS and that the addition of rPHBs mitigates this effect. Preliminary confocal microscopy suggests that rPHB1 localizes to both the mitochondria and nucleus in LPS-treated HK2 cells.

**Conclusion:** Though the mechanism of the effect of rPHBs is still under investigation, these data support the anti-inflammatory and protective effects of rPHB observed in our in vivo endotoxemia model and suggest PHBs may have therapeutic potential in acute kidney injury via mitigation of reactive oxygen species in response to LPS-induced TLR activity.

## Poster #24

**Shweta Wani**

Heparin-Coated PAMAM/CpG G10 Nanoparticles Targeting Plasmacytoid Dendritic Cells Promotes Type I Interferon Mediated Immune Activation In Vitro

**Background:** We aim to implement cancer immunotherapy approach (entailing in situ immunization) using novel immunostimulatory nanoparticles (NPs) designed to target (via heparin coating the NP surface) and activate (via unmethylated CpG motifs) plasmacytoid dendritic cells (pDCs). The activation of pDCs promotes the production of type I interferons, especially IFN $\alpha$ , that in turn stimulates the innate and adaptive immune system, culminating in a tumorspecific immune response. The pDCs are a subset of DCs that express high levels of endosomal Toll-like receptor 9 (TLR9) that are receptors for CpG. They express high levels of endosomal Toll-like receptor 9 (TLR9), which binds to unmethylated CpG motifs, triggering downstream signaling and resulting in massive production of IFN- $\alpha$ . We propose that these NPs, opsonized with heparin, can be internalized by pDCs through the heparin-specific BDCA2 receptor, then activate IFN- $\alpha$  production through CpG: TLR9 interactions and ultimately promote an adaptive tumor-specific immune response.

**Objectives:** The objective of this study was to develop and optimize heparin-coated PAMAM/CpG-G10 nanoparticles by varying N/P ratio and heparin coating concentration, characterize their physicochemical properties (particle size, PDI, and zeta potential), and evaluate in vitro immune activation through IFN- $\alpha$  production in human PBMCs.

**Methods:** PAMAM/G10 NPs were prepared by electrostatic complexation between positively charged PAMAM dendrimers and negatively charged unmethylated CpG-A (G10). The formulation was optimized at different nitrogen-to-phosphate (N/P) ratios (1-10). The NPs were then surfacecoated with varying concentrations of heparin (1 $\mu$ g-30 $\mu$ g) to identify the optimal coating density for BDCA2 targeting. Along with heparin, eight other heparin derivatives were also tested as targeting ligands for BDCA2. The formulations were characterized for particle size, polydispersity index (PDI), and zeta potential using dynamic light scattering (DLS). The immunostimulatory potential of the formulations was tested in vitro using human peripheral blood mononuclear cells (PBMCs). The cells were incubated with NPs formulated at different G10 loadings (0.2  $\mu$ g/mL-2.5  $\mu$ g/mL) and heparin concentrations for 20 hours, and IFN- $\alpha$  secretion was quantified by ELISA.

**Results:** The PAMAM/G10 NPs were successfully developed at an optimized N/P ratio of 3. The NPs had a particle size of  $177 \pm 1.53$  nm d. and PDI of  $0.184 \pm 0.12$ , indicating optimal particle size and uniformity. The NPs, when coated with heparin, demonstrated a concentration-dependent shift in zeta potential, with formulations containing 15-30  $\mu$ g of heparin forming NPs below 200 nm d. with PDI less than 0.2 and zeta potential ranging from -10 mV to -24 mV, indicating successful surface coating. When cultured with human PBMCs, NPs with 2.5  $\mu$ g/mL of G10 and 30  $\mu$ g of heparin induced the highest IFN- $\alpha$  secretion of 1700 pg/mL following 20 hours of incubation. Also, the PBMCs showed increased IFN- $\alpha$  secretion in the presence of higher concentrations of heparin compared to lower concentrations. Other heparin derivatives did not stimulate as much IFN- $\alpha$  production as heparin-coated particles.

**Conclusion:** The results suggest that at higher heparin concentrations, the heparin-coated PAMAM-G10 NPs can promote type I interferon-mediated immune activation in vitro. Among the ligands evaluated, heparin demonstrated the greatest ability to enhance IFN- $\alpha$  production, supporting its potential as a targeting ligand for BDCA2-mediated delivery of TLR9 agonist to pDCs.

## Poster #25

**Emily Jansen**

An Engineered Mixed Microbial Culture for Self-Sustained 1,4-Dioxane Bioremediation

**Background:** 1,4-Dioxane is a cyclic ether that is used as a stabilizer for chlorinated solvents, (e.g., 1,1,1-trichloroethane (TCA) and trichloroethene (TCE)) in many industrial processes. It is an emerging contaminant of particular concern, as it is classified as a probable human carcinogen by the US EPA. Because of its high solubility and stability, dioxane plumes are often large and dilute. Biodegradation with dioxane-metabolizing bacteria is a promising, cost-effective strategy for treating large and dilute dioxane plumes.

**Objectives:** Our group has identified that *Rhodococcus ruber* 219 can metabolize dilute 1,4-dioxane to below health advisory levels ( $0.35 \mu\text{g/L}$ ) when supplemented with thiamine (Vitamin B1). However, vitamin supplementation has proven difficult in lab and field settings. In response, we have developed an engineering syntrophic mixed culture, combining *R. ruber* 219 with VBI hyper-producing bacteria.

**Methods:** After a preliminary batch screening was completed to examine 1,4-dioxane degradation activity, a flow-through column experiment was conducted to test degradation under continuous flow conditions at field-relevant concentrations ( $<100 \mu\text{g/L}$ ). Sand packed columns were inoculated with either *R. ruber* 219 or the mixed culture, and effluent dioxane was measured over the span of three months. For metagenomic analysis, a MinION flow cell from Oxford Nanopore was used to perform long-read sequencing of the mixed culture. Flye was used for de novo assembly of the metagenome. Taxonomic IDs were assigned using Lemur and GTDBtk for the unassembled reads and assembled bins, respectively. Gene annotation was completed with PGAP.

**Results:** This mixed culture has previously demonstrated the ability to completely degrade initial 1,4-dioxane ( $500 \text{ mg/L}$ ) to below detection limits ( $0.05 \mu\text{g/L}$ ) without additional VBI supplements. In the flow-through columns, the mixed culture achieved near-complete 1,4-dioxane degradation by day 25 of the experiment, reducing mean concentrations from  $101.4 (\pm 11.2) \mu\text{g/L}$  to  $2.3 (\pm 3.4) \mu\text{g/L}$ . The resulting metagenomic assemblies identified four different genera (*Rhizobium*, *Rhodococcus*, *Microbacterium*, and *Cupriavidus*) present in the culture, including an exact match to *R. ruber* 219. Further analysis revealed two monooxygenases associated with 1,4-dioxane degradation: the previously characterized *thmA*, located on a high-copy plasmid, and *prmA*, located on the chromosome. The presence of both *thmA* and *prmA* may provide multiple routes for the microbe to initiate 1,4-dioxane degradation and is novel for dioxane-metabolizing bacteria. Additionally, analysis of thiamine biosynthesis genes suggests a potential cross-feeding mechanism in which *R. ruber* 219 scavenges a thiamine precursor produced by *Cupriavidus* to support its thiamine biosynthesis.

**Conclusion:** Together, these results demonstrate a promising approach for 1,4-dioxane bioremediation that eliminates the need for vitamin supplementation in the field. This also addresses the need for an inexpensive and scalable solution for in situ 1,4-dioxane treatment.

## Poster #26

**Elizabeth Walker**

Synthetic biology of non-conventional *Cupriavidus necator* strains through enhanced recombineering for improving PHA production

**Background:** *Cupriavidus necator* is a highly efficient producer of polyhydroxybutyrate (PHB), a biodegradable polymer, capable of accumulating up to 80% of its dry cell weight. However, the metabolic engineering of this bacterium for enhanced production traits is currently hindered by its low recombineering efficiency. Improving the genetic engineering toolkit for this organism is critical for engineering desired traits and developing superior strains for industrial-scale bioplastics production.

**Objectives:** This research aims to accelerate the efficiency of genetic engineering in *C. necator* by utilizing inducible heterologous recombineering machinery. Specific goals include evaluating an inducible Cas9 system as a counter-selection marker and using these recombineering systems to simultaneously knock out PHB depolymerization and integrate a new glucose utilization pathway into the *phaZ1* locus of the genome.

**Methods:** We utilized a dual approach evaluating a lambda-Red recombineering system modified for expression in *C. necator*, alongside a separate system using bacteriophage recombinases Che9c60 and Che9c61. Additionally, we engineered an arabinose-inducible Cas9 system to serve as a counter-selection marker. These tools were evaluated for targeted genome integration in both the *C. necator* H16 type-strain and the highly transformable strain 4182.

**Results:** Initial testing revealed that the lambda-Red recombinase was an inefficient system for integrating the inducible Cas9 cassette into the *C. necator* genome. However, we found that the Cas9 enzyme under the arabinose-inducible promoter functions as a highly efficient counter-selection tool in *C. necator* H16, providing a functional method to isolate successful genomic edits.

**Conclusion:** Heterologous recombinases paired with Cas9 counter-selection offer a promising strategy for overcoming the genetic tractability limitations of *C. necator*. By streamlining these genetic engineering workflows, this research paves the way for precise genomic integration of complex metabolic pathways, such as glucose utilization, while simultaneously preventing polymer degradation. Further optimization of this toolkit will enable the development of highly efficient, customizable PHB production platforms across both type and non-type *C. necator* isolates, advancing the commercial viability of sustainable bioplastics.

## Poster #27

**Sophie Granger**

### Single-Molecule Mechanics Reveals Convergent Allostery in Glutamate Racemase

**Background:** Developing better antibiotics requires understanding not just whether a drug inhibits its target, but how that inhibition actually happens. Allosteric inhibitors offer a promising alternative to traditional active-site drugs by targeting less-conserved protein regions, enabling greater pathogen selectivity. Yet the molecular events connecting allosteric binding to disrupted enzyme function remain poorly understood. Here, we investigate two chemically distinct inhibitors, Compound A and Compound 2, targeting separate allosteric pockets in *Helicobacter pylori* glutamate racemase (HpMurl), an enzyme essential for bacterial cell-wall synthesis, and a mutant previously selected for resistance to Compound A.

**Objectives:** We aimed to uncover the physical mechanism connecting these distinct inhibitor-binding sites, determine how inhibition changes the behavior of individual HpMurl molecules, and investigate how the resistance-associated E151K mutation alters this response.

**Methods:** Using an integrated experimental and computational approach, we characterized HpMurl dimerization, mechanical stability, and collective molecular motions. Mass photometry quantified inhibitor-dependent dimerization across two *H. pylori* strain variants and the E151K mutant. Optical-tweezer force ramps measured unfolding and maximum pulling forces, while separate substrate-only force clamps tracked reversible extension changes. Equilibrium simulations characterized collective motions and intermonomer correlations while steered simulations followed contact rearrangements during forced dimer separation.

**Results:** Despite different baseline assembly distributions, both wild-type strain variants shifted toward dimer with either inhibitor. Surprisingly, both inhibitor-containing tethers unfolded and ruptured at lower maximum pulling forces. In substrate-only force-clamp recordings, dimers repeatedly transitioned between distinct extension states, revealing reversible motions within the uninhibited assembled enzyme. Equilibrium simulations showed that Compound A dampened fluctuations both near its own cryptic pocket and at the distant Pro147-containing interface region implicated in Compound 2 binding. It also weakened coordinated motion between monomers, reducing variance along the dominant collective motions by approximately 51%. During steered simulated separation, Compound A shifted native-contact loss to earlier pulling stages and rearrangements involving the E151-K117' salt bridge and Pro147-containing groove connected the allosteric regions through a shared contact network. The E151K mutant retained catalytic activity and a broad secondary-structure signature but showed substantially reduced dimerization, altered baseline dynamics, and redistributed intermonomer contacts. Importantly, E151K did not eliminate the simulated inhibitor response; it changed how Compound A affected contact rearrangement and physical interface retention during pulling.

**Conclusion:** Our findings reveal an unexpected convergence: two inhibitors targeting different allosteric pockets promote wild-type dimer assembly yet lower the forces required for mechanical perturbation. Compound A's effects on collective motion and contact rearrangement, together with the altered response of E151K, implicate intermonomer communication in allosteric regulation and potentially inhibitor resistance. By connecting protein assembly, reversible single-molecule dynamics, atomistic contact networks, and drug resistance, this work provides new insight into how allosteric inhibitors function and offers a framework for developing more selective antibiotics that exploit protein dynamics.

# Thank you!

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