IOWA | Center for Biocatalysis and Bioprocessing

Advancing Biocatalytic Science through Research and Manufacturing

**September 28th, 2024**

# 2024 CBB Annual Conference Program

# Center for Biocatalysis and Bioprocessing – Annual Conference

September 28th, 2024

College of Pharmacy Building (CPB), University of Iowa, Iowa City

| | | |
|---|---|---|
| *7:30 – 8:15* | Reception and Continental Breakfast – 210 Atrium | |
| *8:15 – 8:30* | **Mark Arnold**: Welcome and Introduction – 210 CPB<br>**Robert Kerns**, Pharmaceutical Sciences and Experimental Therapeutics | |

***Maria Spies Presiding***

| | | |
|---|---|---|
| *8:30 – 9:15* | **John Turchi**, Indiana University | Targeting DNA Damage Response Sensors for First-in-Class Cancer Therapy |
| *9:15 – 10:00* | **Jana Shen**, University of Maryland | AI Models to Discover Covalently Druggable Sites Across the Proteome |
| *10:00 – 10:15* | Break | |

***Ned Bowden Presiding***

| | | |
|---|---|---|
| *10:15 – 11:00* | **Florence Williams**, University of Iowa | Progress Towards Uncovering the Mechanism of Action for Neurotrophic trans-Banglene |
| *11:00 – 11:45* | **Ortwin Ertl**, Annikki GmbH | High-Volume Chemicals from Biomass – By Means of a Unique Fractionation Technology and Enzymatic Reactions |
| *11:45 – 12:00* | Relocation to CPB Atrium | |
| *12:00 – 12:45* | Lunch and Poster Session A | |
| *12:45 – 1:30* | Lunch and Poster Session B | |
| *1:30 – 1:45* | Relocation to seminar room – 210 CPB | |

***Jennifer Fiegel Presiding***

| | | |
|---|---|---|
| *1:45 – 2:30* | **Nicole Hashemi**, Iowa State University | Biophysiological Model of Barriers: Engineered Blood-Brain Barrier and Placental Barrier |
| *2:30 – 2:50* | **Chris Vidmar**, Chemical & Biochemical Engineering | Exploring Interactions between Chemically Produced Bacterial Ghosts and G-Protein Coupled Receptors |
| *2:50 – 3:10* | **Rose Gogal**, Biomedical Engineering | Leveraging Statistical Mechanics for Protein Acid-Base Chemistry Prediction |
| *3:10 – 3:30* | **Ali Khan**, Pathology | Phosphoinositol-3 kinase signaling can be synergistically targeted in sarcoma cancer cells |
| *3:30 – 3:40* | **Michael Schnieders** – Presentation of Student Poster Awards | |
| *3:40 – 3:45* | **Mark Arnold** – Final Comments | |

# Invited Speakers

| **John Turchi**, Indiana University | **Biography** |
|---|---|
| | John J. Turchi, Ph.D. is the Tom and Julie Wood Family Foundation Professor of Lung Cancer Research and a Professor of Medicine and Biochemistry & Molecular Biology at the Indiana University School of Medicine. He is the President, Co-founder, and Chief Scientific Officer (CSO) of NERx Biosciences. Dr. Turchi received his B.S. in Biochemistry from Clemson University and his Ph.D. in Biochemistry from the University of Missouri. He completed a postdoctoral fellowship at the University of Rochester and his research interests lie in DNA damage and repair in the context of cancer therapy. |

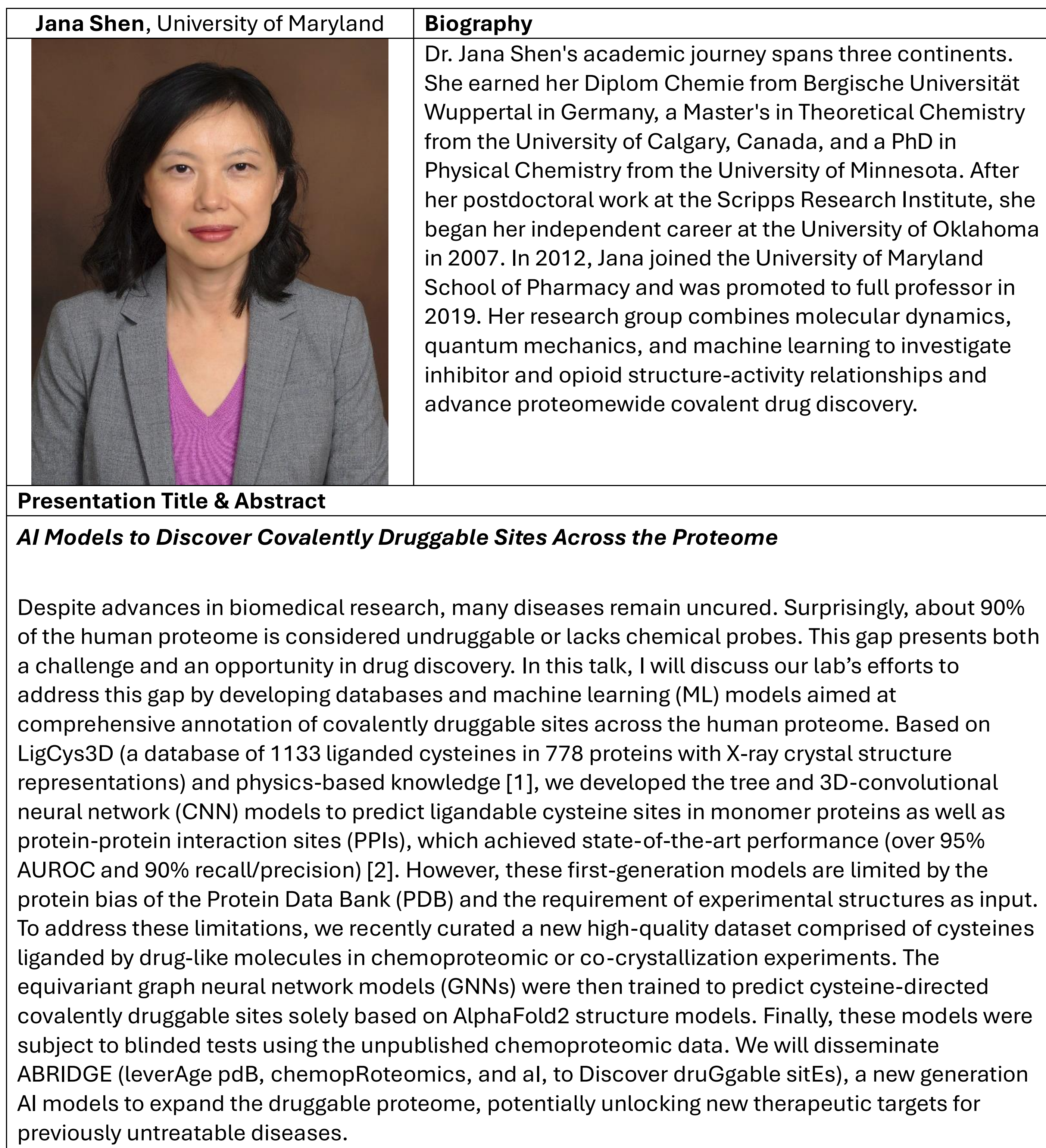
**Presentation Title & Abstract**

***Targeting DNA damage response sensors for first-in-class cancer therapy***


The DNA Damage Response (DDR) is an integrated network of DNA repair and cell signaling pathways that are critical for maintaining genomic stability. The success of PARP inhibitors has spurred the development of numerous DDR-focused therapeutic programs, the majority of which target kinases. However, the clinical outcomes of these approaches have not met expectations. We have pursued the novel approach of intervening upstream of the DDR kinases, targeting specific DDR sensors by developing agents to block protein-DNA interactions as a therapeutic strategy.

The human ssDNA binding protein, replication protein A (RPA), is a critical sensor of ssDNA that activates the DDR in response to replication stress. We have discovered and developed a novel small molecule RPA inhibitor (RPAi) NERx-329 that blocks the RPA-DNA interaction and elicits a state of chemical RPA exhaustion, resulting in in vivo monotherapy anticancer activity. Genetic analyses reveal a series of alterations in DNA repair, recombination, and replication that result in increased sensitivity to RPA inhibition providing insight into the DDR and the potential to target unique patient populations.

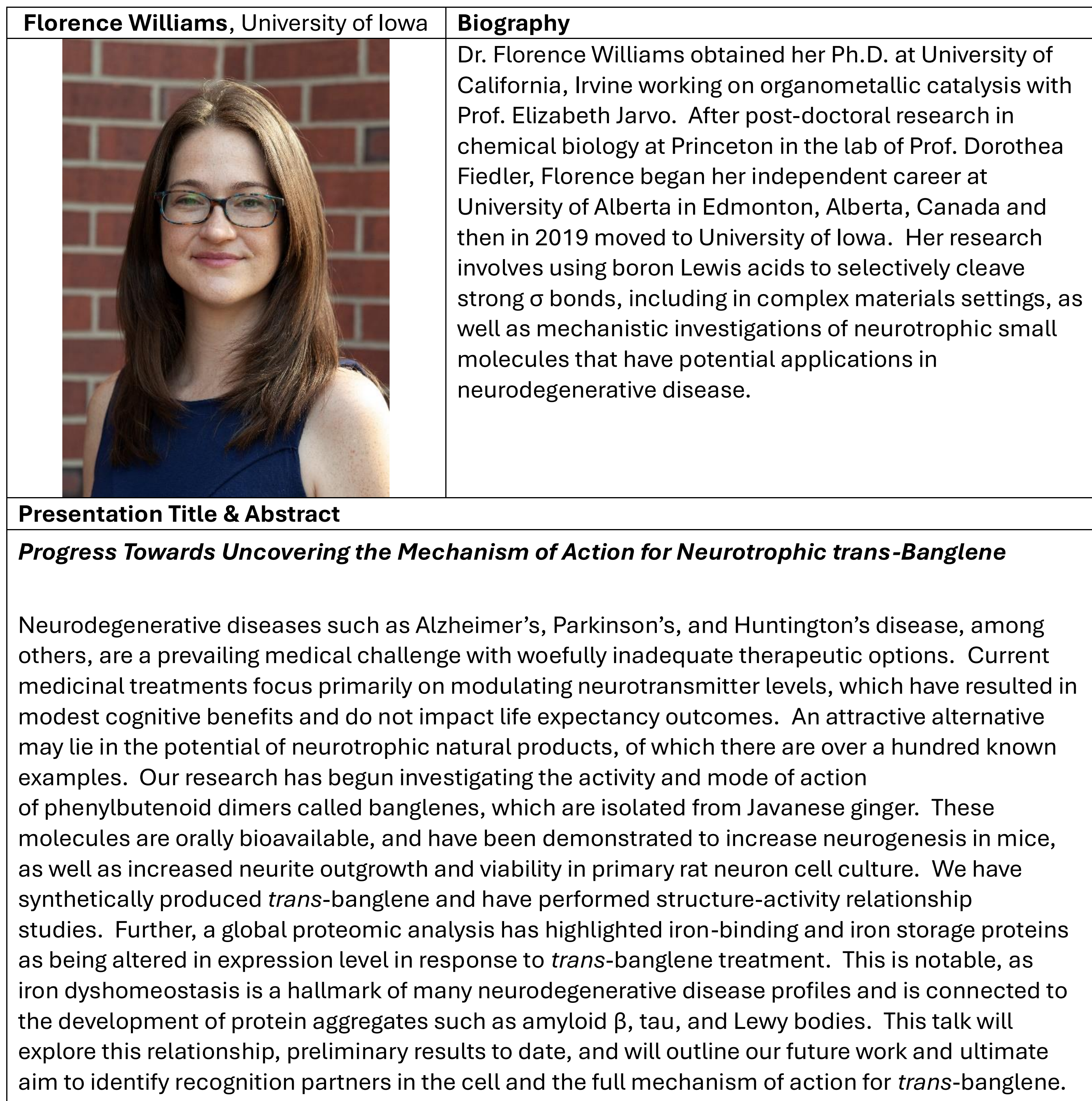
Ku is a sensor of DSBs that binds to DNA ends and activates DNA-PK. Inhibiting DNA-PK is a common strategy to block DSB repair, enhancing the efficacy of therapies like ionizing radiation (IR). We have developed Ku-DNA binding inhibitors (Ku-DBis), which inhibit DSB repair and DNA-PK autophosphorylation resulting in increased sensitivity to IR. We have discovered a novel series of Ku-DBis with improved cellular uptake and bioavailability. These inhibitors show variable anticancer effects across a series of non-small cell lung cancer (NSCLC) cell lines, with ATM-null cells showing the most sensitivity. The efficacy of Ku-DBis assessed alone and in combination with IR in a human xenograft model of NSCLC demonstrates that Ku-DBi treatment blocks IR-dependent DNA-PKcs autophosphorylation, modulates the DDR, and reduces tumor cell proliferation. This work is the first in vivo assessment of an optimized novel Ku-DBi and these data represent a significant advance in the development of Ku-DNA binding inhibitors and their potential therapeutic utility for cancer treatment.

| **Jana Shen**, University of Maryland | **Biography** |
|---|---|
|  | Dr. Jana Shen's academic journey spans three continents. She earned her Diplom Chemie from Bergische Universität Wuppertal in Germany, a Master's in Theoretical Chemistry from the University of Calgary, Canada, and a PhD in Physical Chemistry from the University of Minnesota. After her postdoctoral work at the Scripps Research Institute, she began her independent career at the University of Oklahoma in 2007. In 2012, Jana joined the University of Maryland School of Pharmacy and was promoted to full professor in 2019. Her research group combines molecular dynamics, quantum mechanics, and machine learning to investigate inhibitor and opioid structure-activity relationships and advance proteomewide covalent drug discovery. |

**Presentation Title & Abstract**

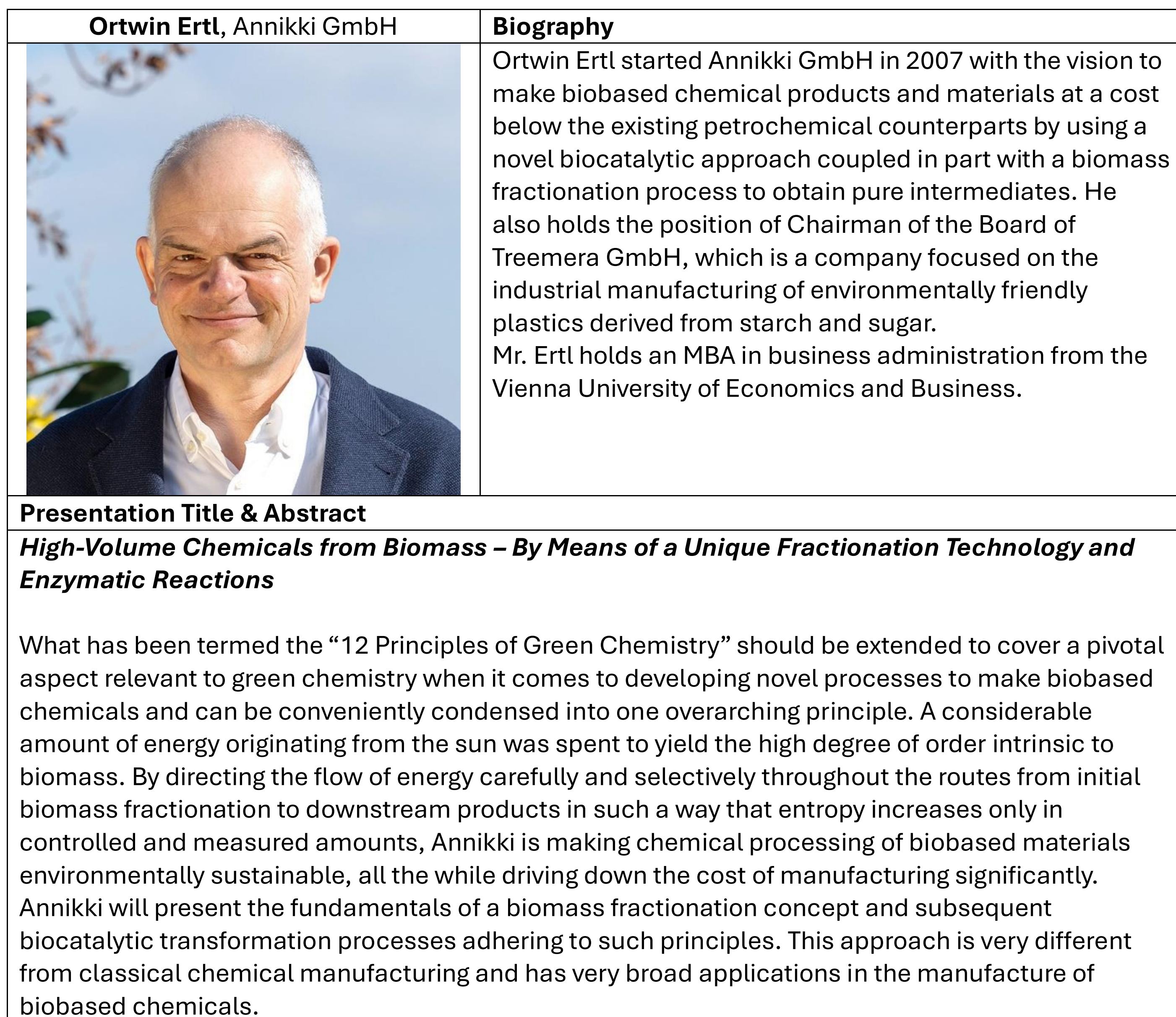
***AI Models to Discover Covalently Druggable Sites Across the Proteome***

Despite advances in biomedical research, many diseases remain uncured. Surprisingly, about 90% of the human proteome is considered undruggable or lacks chemical probes. This gap presents both a challenge and an opportunity in drug discovery. In this talk, I will discuss our lab's efforts to address this gap by developing databases and machine learning (ML) models aimed at comprehensive annotation of covalently druggable sites across the human proteome. Based on LigCys3D (a database of 1133 liganded cysteines in 778 proteins with X-ray crystal structure representations) and physics-based knowledge [1], we developed the tree and 3D-convolutional neural network (CNN) models to predict ligandable cysteine sites in monomer proteins as well as protein-protein interaction sites (PPIs), which achieved state-of-the-art performance (over 95% AUROC and 90% recall/precision) [2]. However, these first-generation models are limited by the protein bias of the Protein Data Bank (PDB) and the requirement of experimental structures as input. To address these limitations, we recently curated a new high-quality dataset comprised of cysteines liganded by drug-like molecules in chemoproteomic or co-crystallization experiments. The equivariant graph neural network models (GNNs) were then trained to predict cysteine-directed covalently druggable sites solely based on AlphaFold2 structure models. Finally, these models were subject to blinded tests using the unpublished chemoproteomic data. We will disseminate ABRIDGE (leverAge pdB, chemopRoteomics, and aI, to Discover druGgable sitEs), a new generation AI models to expand the druggable proteome, potentially unlocking new therapeutic targets for previously untreatable diseases.

<table>
<tr><td>Florence Williams, University of Iowa</td><td>Biography</td></tr>
<tr><td></td><td>Dr. Florence Williams obtained her Ph.D. at University of California, Irvine working on organometallic catalysis with Prof. Elizabeth Jarvo.  After post-doctoral research in chemical biology at Princeton in the lab of Prof. Dorothea Fiedler, Florence began her independent career at University of Alberta in Edmonton, Alberta, Canada and then in 2019 moved to University of Iowa.  Her research involves using boron Lewis acids to selectively cleave strong σ bonds, including in complex materials settings, as well as mechanistic investigations of neurotrophic small molecules that have potential applications in neurodegenerative disease.</td></tr>
<tr><td colspan="2">Presentation Title & Abstract</td></tr>
</table>

***Progress Towards Uncovering the Mechanism of Action for Neurotrophic trans-Banglene***

Neurodegenerative diseases such as Alzheimer’s, Parkinson’s, and Huntington’s disease, among others, are a prevailing medical challenge with woefully inadequate therapeutic options.  Current medicinal treatments focus primarily on modulating neurotransmitter levels, which have resulted in modest cognitive benefits and do not impact life expectancy outcomes.  An attractive alternative may lie in the potential of neurotrophic natural products, of which there are over a hundred known examples.  Our research has begun investigating the activity and mode of action of phenylbutenoid dimers called banglenes, which are isolated from Javanese ginger.  These molecules are orally bioavailable, and have been demonstrated to increase neurogenesis in mice, as well as increased neurite outgrowth and viability in primary rat neuron cell culture.  We have synthetically produced *trans*-banglene and have performed structure-activity relationship studies.  Further, a global proteomic analysis has highlighted iron-binding and iron storage proteins as being altered in expression level in response to *trans*-banglene treatment.  This is notable, as iron dyshomeostasis is a hallmark of many neurodegenerative disease profiles and is connected to the development of protein aggregates such as amyloid β, tau, and Lewy bodies.  This talk will explore this relationship, preliminary results to date, and will outline our future work and ultimate aim to identify recognition partners in the cell and the full mechanism of action for *trans*-banglene.

| **Ortwin Ertl**, Annikki GmbH | **Biography** |
| --- | --- |
|  | Ortwin Ertl started Annikki GmbH in 2007 with the vision to make biobased chemical products and materials at a cost below the existing petrochemical counterparts by using a novel biocatalytic approach coupled in part with a biomass fractionation process to obtain pure intermediates. He also holds the position of Chairman of the Board of Treemera GmbH, which is a company focused on the industrial manufacturing of environmentally friendly plastics derived from starch and sugar.<br>Mr. Ertl holds an MBA in business administration from the Vienna University of Economics and Business. |

**Presentation Title & Abstract**

***High-Volume Chemicals from Biomass – By Means of a Unique Fractionation Technology and Enzymatic Reactions***

What has been termed the “12 Principles of Green Chemistry” should be extended to cover a pivotal aspect relevant to green chemistry when it comes to developing novel processes to make biobased chemicals and can be conveniently condensed into one overarching principle. A considerable amount of energy originating from the sun was spent to yield the high degree of order intrinsic to biomass. By directing the flow of energy carefully and selectively throughout the routes from initial biomass fractionation to downstream products in such a way that entropy increases only in controlled and measured amounts, Annikki is making chemical processing of biobased materials environmentally sustainable, all the while driving down the cost of manufacturing significantly. Annikki will present the fundamentals of a biomass fractionation concept and subsequent biocatalytic transformation processes adhering to such principles. This approach is very different from classical chemical manufacturing and has very broad applications in the manufacture of biobased chemicals.

| **Nicole Hashemi**, Iowa State University | **Biography** |
| --- | --- |
| | Dr. Nicole Hashemi is an Associate Professor in the Department of Mechanical Engineering at Iowa State University. Her research interests are in the areas of microfluidics, biomedical microdevices, and materials. She is the recipient of the 2023 NSF Mid-CAREER Advancement Award for her placenta-on-a-chip project. She has also been the recipient of the NSF EAPSI Fellowship, NRC Postdoctoral Fellowship, NRL Research Publication Award, Big 12 Faculty Fellowship Award, and ISU Early Career Engineering Faculty Research Award. Hashemi has also been selected as both the National Academy of Engineering US Frontiers of Engineering and the National Academy of Sciences Kavli Frontiers of Science. She is a Fellow of the American Society of Mechanical Engineers and a Fellow of the Royal Society of Chemistry. She is an Associate Editor of npj Women's Health and ASME Journal of Biomechanical Engineering. |

**Presentation Title & Abstract**

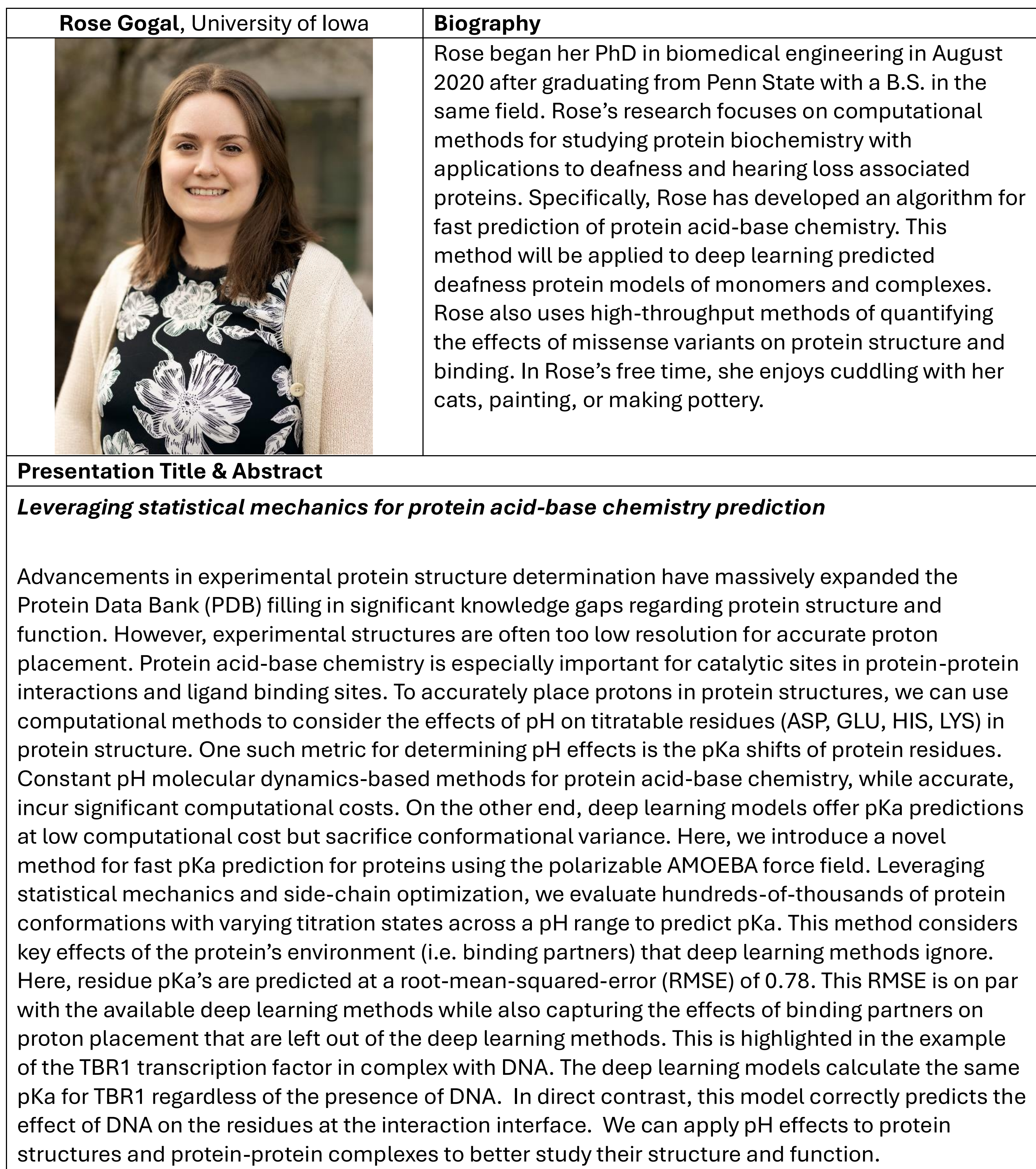
***Microphysiological Model of Barriers: Engineered Blood-Brain Barrier and Placental Barrier***

We have created engineered microstructures to mimic the blood-brain barrier (BBB) and placental barrier.

The BBB is a highly selective semipermeable membrane made up of endothelial cells that separates blood from the brain's extracellular fluid, thus regulating the chemicals that reach the brain from the circulatory system. The BBB breaks down with age, and its disruption plays a role in many age-related disorders, primarily neurodegenerative diseases (NDs). NDs are a group of diseases that entail progressive degeneration of the structure and function of neurons. Neurons do not generally regenerate after they are damaged, so degeneration may lead to impairments in cognition, motor skills, and sensory functions. There is currently no cure for NDs, but treatments focus on managing symptoms and improving quality of life. Research is ongoing to better understand the underlying mechanisms of these disorders. The lack of an accurate in-vitro BBB model leads to a poor understanding of the breakdown that occurs with age, which has the potential to lead to NDs. Likewise, the lack of research on electrical properties within the BBB and waste elimination tactics for in-vitro models are other important areas to be investigated. We have successfully created an innovative 3-D cell-culture platform that mimics the tubal structure found in the BBB. This platform is capable of hosting neural cell lines, such as human brain endothelial and astrocyte cells.

Due to the particular structure and functionality of the placenta, most current human placenta drug testing methods are limited to animal models, conventional cell testing, and cohort/controlled testing. Previous studies have produced inconsistent results due to physiological differences between humans and animals and limited availability of human and/or animal models for controlled testing. To overcome these challenges, a placenta-on-a-chip system has been developed for studying the exchange of substances to and from the placenta. Xenobiotic compound transport across the placental barrier is studied due to the daily exposure of pregnant individuals to these materials.

<table>
<tr><td>Chris Vidmar, University of Iowa</td><td>Biography</td></tr>
<tr><td> </td><td>Christopher Vidmar is a PhD candidate in the Department of Chemical and Biochemical Engineering at the University of Iowa. His research focuses on developing innovative drug delivery systems that harness bacteria’s natural targeting abilities for localized treatment, aiming to enhance therapeutic effectiveness. He completed a fellowship with the CBB and was recognized for his research impact by winning the College of Engineering's Three Minute Thesis competition. His internship at Boston Scientific provided crucial experience in medical device development. By integrating his expertise in both drug delivery and medical devices, Christopher aims to advance cutting-edge medical technologies.</td></tr>
<tr><td colspan="2">Presentation Title & Abstract</td></tr>
<tr><td colspan="2">Exploring interactions between chemically produced bacterial ghosts and G-protein coupled receptors (GPCRs)<br><br>Bacteria display surface components that can interact with receptors found within the body. These surface components are known to interact with G-protein coupled receptors (GPCRs), a superfamily of transmembrane receptors that are often overexpressed in certain disease conditions. Interactions between bacterial surface components and GPCRs can facilitate bacterial adherence and internalization or can regulate an immune response by hijacking receptor signaling. We hypothesize that by exploiting these bacteria-GPCR interactions, we can develop effective targeted drug delivery carriers. However, the extent that these interactions occur remain unclear since conventional methods only investigate one receptor-ligand pair at a time. Our approach is to produce bacterial ghosts that will allow us to use high-throughput screening techniques to explore interactions between bacterial surface components and hundreds of GPCRs. Ghost bacteria are non-living bacterial cells devoid of their internal components with a fully intact membrane. This project ultimately aims to use these bacterial ghosts as a drug delivery vehicle targeting GPCRs. Bacterial ghosts from two commensal E. coli strains and a probiotic E. coli strain were chemically produced using dilute concentrations of a cell lysis buffer. Interactions between these ghost bacteria and 314 GPCRs were then explored using a high-throughput screening technique that measures GPCR signaling. Successful bacterial ghost production was confirmed by measuring DNA release and imaging the intact membrane with SEM. A preliminary high-throughput screening with these bacterial ghosts identified interactions that overlap with known bacteria-host pathways and identified previously unexplored interactions. Future work is dedicated to validating initial hits, orthogonal assays to probe the functional response of these interactions, and drug loading/release studies. Overall, this study presents a platform to produce bacterial ghosts and to explore their interactions in a high-throughput manner, allowing us to perform the first high-throughput screen between whole bacteria and hundreds of GPCRs.</td></tr>
</table>

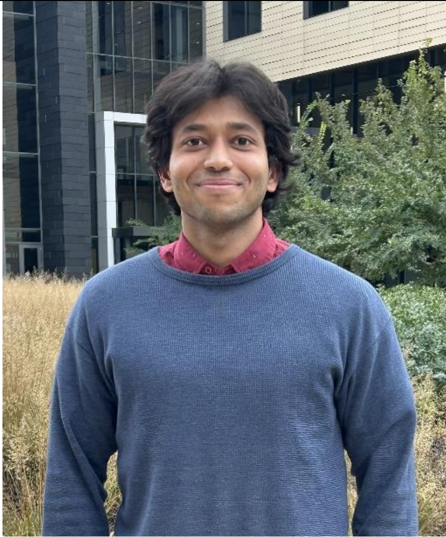
| **Rose Gogal**, University of Iowa | **Biography** |
| --- | --- |
|  | Rose began her PhD in biomedical engineering in August 2020 after graduating from Penn State with a B.S. in the same field. Rose's research focuses on computational methods for studying protein biochemistry with applications to deafness and hearing loss associated proteins. Specifically, Rose has developed an algorithm for fast prediction of protein acid-base chemistry. This method will be applied to deep learning predicted deafness protein models of monomers and complexes. Rose also uses high-throughput methods of quantifying the effects of missense variants on protein structure and binding. In Rose's free time, she enjoys cuddling with her cats, painting, or making pottery. |

**Presentation Title & Abstract**

***Leveraging statistical mechanics for protein acid-base chemistry prediction***

Advancements in experimental protein structure determination have massively expanded the Protein Data Bank (PDB) filling in significant knowledge gaps regarding protein structure and function. However, experimental structures are often too low resolution for accurate proton placement. Protein acid-base chemistry is especially important for catalytic sites in protein-protein interactions and ligand binding sites. To accurately place protons in protein structures, we can use computational methods to consider the effects of pH on titratable residues (ASP, GLU, HIS, LYS) in protein structure. One such metric for determining pH effects is the pKa shifts of protein residues. Constant pH molecular dynamics-based methods for protein acid-base chemistry, while accurate, incur significant computational costs. On the other end, deep learning models offer pKa predictions at low computational cost but sacrifice conformational variance. Here, we introduce a novel method for fast pKa prediction for proteins using the polarizable AMOEBA force field. Leveraging statistical mechanics and side-chain optimization, we evaluate hundreds-of-thousands of protein conformations with varying titration states across a pH range to predict pKa. This method considers key effects of the protein's environment (i.e. binding partners) that deep learning methods ignore. Here, residue pKa's are predicted at a root-mean-squared-error (RMSE) of 0.78. This RMSE is on par with the available deep learning methods while also capturing the effects of binding partners on proton placement that are left out of the deep learning methods. This is highlighted in the example of the TBR1 transcription factor in complex with DNA. The deep learning models calculate the same pKa for TBR1 regardless of the presence of DNA. In direct contrast, this model correctly predicts the effect of DNA on the residues at the interaction interface. We can apply pH effects to protein structures and protein-protein complexes to better study their structure and function.

**Ali Khan**, University of Iowa



**Biography**

Ali Khan was born and raised in Karachi, Pakistan and moved to New York for his undergraduate degree. Given the interdisciplinary nature of today's research, he explored a variety of fields, including biochemistry, molecular and cell biology, bioinformatics, and even electrical engineering at SUNY Oswego during his undergraduate education. Given his strong interest in cancer signaling, he joined Dr. Tanas' lab to understand the role of PI3 kinase signaling and Hippo pathway in sarcomagenesis. His long-term goal is to therapeutically target these pathways to improve patient care.

**Presentation Title & Abstract**

***Phosphoinositol-3 kinase signaling can be synergistically targeted in sarcoma cancer cells***

Sarcomas are cancers primarily arising from connective tissues. They represent over 50 histological types with few effective targeted therapies. Hyperactivation of PI3K, a serine/threonine kinase crucial for cell survival and proliferation is commonly observed in sarcomas due to deletion of PTEN in approximately 20-30% of sarcomas. Thus, targeting PI3K may serve as an effective potential treatment for these sarcomas. The mTORC1 complex has been well-established as a key downstream effector of PI3K signaling. We have generated additional data suggesting that YAP/TAZ represent key transcriptional effectors of PI3K signaling. mTORC1 is a regulator for protein synthesis and cell survival while YAP/TAZ, downstream targets of Hippo pathway, are transcriptional coactivators that promote sarcomagenesis and metastasis. How these two pathways coordinately mediate oncogenic PI3K signaling has been poorly studied representing a significant gap in knowledge. Tissue microarray analysis showed that approximately 46% of samples display loss of PTEN protein expression and 38% of undifferentiated pleomorphic sarcoma (UPS) also displayed loss of PTEN expression. Various sarcoma cell lines also exhibit elevated phosphorylation of the PI3K target, Akt, as well as loss of PTEN expression. Conditional knock-out of both Wwtr1 (Taz) and Yap improves survival in a Trp53fl/fl Ptenfl/fl mouse model of UPS. RNA-Seq analysis performed on tumors from conditional mice reveals subsets of genes regulated by Wwtr1, Yap, and both Wwtr1 and Yap. Interestingly, pathway analysis showed alteration of Hippo and mTORC1 pathway in Trp53fl/fl Ptenfl/fl Wwtr1fl/fl Yapfl/fl mice tumor samples. Upon treatment with mTORC1 inhibitor, Everolimus, and YAP/TAZ inhibitor, IK-930, and we showed that monotherapy had a very modest effect on cell proliferation and growth both in vivo and in vitro. However, upon combination therapy with Everolimus and IK-930, synergistic activity was seen both in vitro and in vivo seen by significant reduction in cell growth and proliferation. The combination therapy also showed significant reduction in tumor burden in mice relative to monotherapy controls.

Our data supports the model that TAZ/YAP are key transcriptional co-activators downstream of PI3K signaling. This work suggests that TAZ/YAP as well as mTORC1 mediate oncogenic PI3K signaling. We showed that dual inhibition of mTORC1 and YAP/TAZ-TEAD can serve as an effective therapy in PI3K-activated sarcomas.

# Poster Presentations

| Poster No. | Presenter | Poster Title |
|---|---|---|
| 1 | Heba Abuzenah | *Selective Deracemization of Tetralol to Tetralone Using AaeUPO Peroxygenase: Bridging Nature and Industry* |
| 2 | Sarah Jordan | *Biochemical and Single-Molecule Studies of Translesion Synthesis Polymerases* |
| 3 | Tyler Woodward | *Unraveling the Mechanism of Action of Tardigrade Damage Suppressor, Dsup* |
| 4 | Joseph Correa | *Delivering Epinephrine Via Dissolving Microneedles and Topical Gels* |
| 5 | Umed Singh | *Prodrug forms of a phosphonate ligand for butyrophilin 3A1* |
| 6 | Mary Schad | *Design and synthesis of insulin sensitizers for the treatment of Type II Diabetes* |
| 7 | Pornpoj "Jay" Phruttiwanichakun | *Local delivery of CST6 mRNA for bone regeneration* |
| 8 | Paras Gaur | *Observing PARP1 interactions with G-quadruplexes and PARylation at single-molecule level* |
| 9 | Yan Xu | *In Situ Immunization with a TLR9 Agonist Virus-Like Particle to Activate Immune Response Against Leukoplakia Progression* |
| 10 | Cynthia Okafor | *Exploring Bacterial Cellulose and Nanocellulose for Biomanufacturing* |

| | | |
|---|---|---|
| **11** | **Eman Kamel** | ***Forced degradation of cell-based therapeutic products using various stresses and use of flow imaging microscopy for identification of damaged cells and cell debris.*** |
| **12** | **Siddhanth Hejmady** | **A Comparative Study of Agitation-Induced Aggregation of Monoclonal Antibodies via Horizontal Shaking and Orbital Shaking** |
| **13** | **Zoe Kramin** | ***Development of Real-Time Predictions for Organic Slurry Composition and Polysaccharide Utilization Loci Metabolism*** |
| **14** | **Mortezaali Razzaghi** | ***CRYO-EM STRUCTURAL STUDIES OF HUMAN RAD52 DNA REPAIR PROTEIN*** |
| **15** | **Ramkrishna Sen** | ***Co-loaded Nanoparticle Formulation of Encorafenib and Trametinib for Targeting BRAF V600E in Colon Cancer*** |
| **16** | **Apurva Dusane** | ***Nanomedicine for Fuchs endothelial corneal dystrophy*** |
| **17** | **Sophie Granger** | ***Human hnRNPA1 reorganizes telomere-bound Replication Protein A*** |
| **18** | **Rebecca Splitt** | ***Investigating E-cadherin Mediated Force Transmission*** |
| **19** | **Hesham Refaat** | ***Challenges with Removal and Addition of Polysorbates From/To Formulations of Monoclonal Antibodies*** |
| **20** | **Maclaine Putney** | ***Diffuse reflectance spectroscopy for non-destructive composition measurements of algal biofilm*** |
| **21** | **Samuel Yu** | ***Akt inhibition: potential therapeutic target for PI3K-driven sarcomas?*** |

| | | |
|---|---|---|
| 22 | Hossein Zare | *In Vitro Dispersion of Pseudomonas aeruginosa Biofilms* |
| 23 | Souradip Sinha | *The ATAC HAT complex is a critical epigenetic complex driving sarcomagenesis* |
| 24 | *Adam Benmoussa* | *The Crystallization and Structure of Human Alternative Replication Protein A* |
| 25 | Mohammad Alnatour | *Active Targeting of Colorectal Cancer Using Chemotherapy-loaded Nanoparticles Functionalized with Folate Receptor-α (FRα) Ligand, Pemetrexed* |

# Even number poster = Session A 12:00 – 12:45

# Odd number poster = Session B 12:45 – 1:30

## Poster #1

| Heba Abuzenah | Selective Deracemization of Tetralol to Tetralone Using AaeUPO Peroxygenase: Bridging Nature and Industry |
|---|---|

Background: This research explores the deracemization of racemic mixtures to obtain single enantiomers, which is critical due to the industrial and pharmaceutical relevance of enantiomerically pure compounds.

Objectives: The primary objective is to evaluate the feasibility of achieving deracemization by first oxidizing alcohols to ketones and subsequently reducing them to single enantiomers. Specifically, we aim to deracemize tetralols to tetralones, given their industrial importance.

Methods: We utilized the Agrocybe aegerita (AaeUPO) peroxygenase enzyme to oxidize specific tetralols to tetralones in a phosphate buffer (50 mM, pH 7.0) with hydrogen peroxide and acetonitrile at 30°C and 180 rpm for 5 minutes. The oxidation products were analyzed using GC-MS and chiral gas chromatography to determine their stereochemical purity and configuration. Following oxidation, a W110V mutant of the TeSADH enzyme was employed for enantioselective reduction, achieving conversion rates exceeding 99%.

Results: The oxidation process using AaeUPO peroxygenase successfully converted tetralols to tetralones, which were analyzed and confirmed to have high stereochemical purity. Subsequent reduction with the W110V mutant of TeSADH enzyme resulted in enantioselective reduction with conversion rates exceeding 99%.

Conclusion: This study demonstrates the feasibility of a one-pot oxidation and reduction process for obtaining enantiomerically pure compounds. The successful deracemization of tetralols to tetralones, alongside other ketones, highlights the potential industrial application of this method, providing a sustainable and efficient approach for producing enantiomerically pure compounds.

## Poster #2

| Sarah Jordan | ***Biochemical and Single-Molecule Studies of Translesion Synthesis Polymerases*** |
|---|---|

Background: DNA damage is a serious threat to genome stability. Damage can occur during DNA replication often leading to replication fork stalling, which can cause many DNA mutations and eventual cell death. Therefore, eukaryotic cells have evolved to escape stalled replication forks through DNA damage bypass mechanisms. One of these mechanisms is known as Translesion Synthesis (TLS) which utilizes specialized polymerases (TLS polymerases) evolved to incorporate across DNA damage. To initiate, TLS requires the mono-ubiquitylation of the Proliferating Cellular Nuclear Antigen (PCNA), which acts as the master regulator of replication. The mono-ubiquitylation is catalyzed by a complex of proteins, known as Rad6 and Rad18 (Rad6/18). After mono-ubiquitylation occurs, specialized TLS polymerases, such as polymerase eta (η), are recruited to the replication fork. Once DNA damage bypass has occurred, the TLS polymerase dissociates, and the replicative polymerase engages with PCNA to restore normal (undamaged) replication.

Objectives: Although the general mechanisms are understood, it is unclear the role Rad6/18 plays in TLS. While it has been suggested that mono-ubiquitylation of PCNA initiates TLS, preliminary mass photometry data show polymerase and Rad6/18 form a complex. We aim to biochemically and structurally understand this interaction. Additionally, the structure of the bypass complex is unclear. Therefore, we seek to gain insight into the functional consequences of these interactions and the composition of the bypass complex during TLS.

Methods & Results: Here, we utilize steady-state kinetics to investigate the biochemical effects of the interaction between Rad6/18 and polymerase η. We found that this interaction increases polymerase η's catalytic efficiency – the ability of the polymerase to extend DNA. Additionally, we are utilizing C-Trap to the dynamic complexes of TLS through single molecule studies by use of C-Trap. In which we are working to consistently load PCNA onto our DNA substrate.

Conclusions**:** Interactions with Rad6/18 may dictate the selection of the TLS polymerases and additionally increase the catalytic efficiency. An increased catalytic efficiency may allow a TLS polymerase to bypass damage more quickly, preventing replication fork collapse. Information from these studies will be important in providing a comprehensive understanding of TLS which could facilitate the development of drugs to prevent genome instability and/or increase the potency of chemotherapy agents that block DNA replication such as cisplatin.

## Poster #3

| **Tyler Woodward** | ***Unraveling the Mechanism of Action of Tardigrade Damage Suppressor, Dsup*** |
|---|---|

Background: Radiation exposure is detrimental to living organisms. Generally, high energy radiation exposure leads to DNA damage – leading to genome instability, premature aging, and many other types of diseases. For this reason, it is important to study the cellular mechanisms that protect from radiation exposure. Tardigrades have been a model organism for studying radiation exposure. Tardigrades are microscopic invertebrates that have the ability to survive immense levels of radiation. Tardigrade tolerance to radiation has been attributed to a Tardigrade-unique protein, Dsup (Damage suppressor). Previous work has demonstrated that Dsup protects from ionizing radiation, and UV radiation. Cells expressing Dsup also have less DNA damage when exposed to reactive oxygen species (ROS). ROS is a byproduct from ionizing radiation that leads to DNA damage. Importantly, HEK-293T (human embryonic kidney) cells expressing Dsup have increased radiotolerance and less DNA damage upon exposure to radiation.

Objective: While previous studies have detailed the effect of expressing Dsup in several other organisms (including *Drosophila, Saccharomyces,* rice plants, and tobacco plants), the mechanism of action remains unknown. This work seeks to elucidate the mechanism of action in which Dsup confers protection from radiation. The central hypothesis is that Dsup has two modes of action: (1) physical protection from ROS, and (2) induced conformational change to DNA bases to protect from UV radiation.

Methods: To evaluate the validity of this central hypothesis, a combination of experimental approaches was utilized, including Mass Photometry, Biolayer Interferometry (BLI), Small-angle X-ray Scattering (SAXS), and Brownian Dynamic (BD) simulations.

Results: Dsup was purified from *E. coli* cells through heterologous expression. Utilizing mass photometry, the predominant oligomeric state of Dsup is monomeric with approximately 10% forming homodimers. Furthermore, we utilized biolayer interferometry to describe the affinity of Dsup to several different DNA substrates. We concluded the following: (1) Dsup appears to bind with an occlusion length between 30-40 base pairs, (2) the affinity toward single- and double- stranded DNA is within 2-orders of magnitude, and (3) that the C-terminal (aa. 355-445) is sufficient to bind to DNA. Consequentially, the C-terminal alone binds with significantly lower affinity which suggests the N-terminal is required for low nanomolar affinity. Lastly, we utilized a full-ensemble approach to describe the repertoire of structures possible for Dsup. We found that Dsup is extremely disordered and maintains an extended conformation.

Conclusion: Taken together, these findings describe the biochemistry behind Dsup binding to DNA. Overall, this work significantly contributes to the field's understanding of Dsup. Further experiments are required to fully elucidate the mechanism, but this work is crucial for creating a model for the mechanism of action. Currently, we are working to solve the structure of a Dsup bound to a nucleosome utilizing Cryo-EM. With a bound structure, we hope to force Dsup into one conformation. This structure will provide many details for how Dsup binds to nucleosomes.

## Poster #4

| Joseph Correa | ***Delivering Epinephrine Via Dissolving Microneedles and Topical Gels*** |
|---|---|

Background: Epinephrine is typically administered intravenously or intramuscularly during acute anaphylaxis to stabilize patients until medical help arrives. These injections cause pain, run risks of infections, and are often less preferred by patients. Dissolving microneedles (dMNs) bypass the stratum corneum in the skin, allowing a patient-friendly method to painlessly self-administer medication. Furthermore, topical gels can produce a sustained drug release.

Objectives: The goal of this project is to combine dMNs with a topical gel to achieve a rapid and sustained release of epinephrine.

Methods: In this study, dMNs were formulated from either hyaluronic acid (HA, 10% and 15% w/v), a hyaluronic acid-polyvinylpyrrolidone mixture (4% HA-30% PVP w/v), or pullulan (PL, 15% w/v). PL (15% w/v) and HA-PVP (4%:30% w/v) were used to formulate dMNs with 0.1% (w/v) epinephrine to compare the mechanical properties of blank and epinephrine-loaded dMNs. Molds of 600 or 800 μm needles in 10x10 arrays were used to load each formulation and cast dMNs. The physical characteristics of the dMNs were evaluated under a microscope. Axial compression tests were conducted with a rotational rheometer to assess the mechanical strength of the dMNs. Additionally, 0.1% (w/w) epinephrine was added with 1, 2, and 3% (w/w) hydroxyethyl-cellulose (HEC) and 15% (w/w) PL to formulate topical gels. Franz diffusion cells and high-performance liquid chromatography (HPLC) were utilized to quantify the in vitro drug release of epinephrine from the gels.

Results: The dMNs made with 15% w/v PL showed the best dMN forming ability and strong mechanical properties, with a mean percent needle height reduction of 15.2 ± 5.4% (n=18) for the blank dMNs and 13.0 ± 2.9% (n=18) for the epinephrine-loaded dMNs. Additionally, these dMNs withstood up to 20 N of force without cracking. HA alone, both 10% and 15% w/v, produced dMNs with fractures before they were removed from the mold. Pairing HA with PVP yielded dMNs without fractures, but the dMNs were inconsistently formed and cracked under as little as 3.4 N of force. The 2% w/w HEC gel displayed the highest release of epinephrine with 87.1% released after 1 hour. 15% PL released more than the 1% and 3% HEC gels with 77.5% of the epinephrine after 1 hour.

Conclusion: These results demonstrate the potential for epinephrine to be delivered via dMNs and gels for a rapid and sustained release in emergency situations. 15% PL formed the best dMNs with strong mechanical properties that are more than sufficient for applying dMNs to the skin. The HA-PVP dMNs fractured under low levels of force. Testing additional concentrations of each component may improve the mechanical properties of the dMNs. The 2% HEC gel achieved a high, sustained release over 1 hour. The combination of a 15% PL dMN with strong mechanical properties and a 2% HEC topical gel that can achieve a sustained release of epinephrine could prove to be an ideal pairing to achieve a rapid and sustained release of epinephrine for the treatment of acute anaphylaxis to stabilize patients long enough for additional medical help to arrive.

## Poster #5

| Umed Singh | ***Prodrug forms of a phosphonate ligand for butyrophilin 3A1***<br>***Umed Singh, Girija Pagwe, Sarita Rani, Chia-Hung Christine Hsiao Andrew J. Wiemer, David F. Wiemer*** |
|---|---|

Background: Butyrophilin 3A1 (BTN3A1) is an immune co-receptor that binds molecules such as (E)-4-hydroxy-3-methyl-but-2-enyl diphosphate (HMBPP) leading to an immune response by Vgamma9Vdelta2 T cells. Because HMBPP is highly charged and metabolically unstable, prodrugs may be needed to overcome these liabilities but prodrugs themselves may be limited by slow payload release or low plasma stability. In earlier work we have prepared a several prodrugs of a phosphonate analog of HMBPP and characterized their ability to stimulate these T cells and some have displayed sub-nanomolar potency.

Objectives: We have continued these investigations in search of compounds that combine high potency with significant serum stability. Because release of the drug cargo is reliant upon an esterase mediated hydrolysis of the prodrug, serum stability might be achievable if the phosphonate is (relatively) stable to serum esterases. However, the prodrug must be readily cleaved by intracellular enzymes for effective drug delivery.

Methods: To identify effective prodrug forms of a phosphonate agonist of BTN3A1, we have prepared a novel set of diesters bearing one aryl and one acyloxymethyl group. The compounds were evaluated for their ability to stimulate Vγ9Vδ2 T cell proliferation, increase production of interferon γ, resist plasma metabolism, and internalize into leukemia cells. These bioassays have revealed that varied aryl and acyloxyalkyl groups can decouple plasma and cellular metabolism and have a significant impact on bioactivity and stability, including some with sub-nanomolar potency.

Results: In some cases, potency for stimulation of T cell proliferation and cytokine production is still sub-nanomolar while increased stability to serum esterases also has been observed.

Conclusion: Some of the compounds tested showed significantly enhanced plasma stability relative to the acyloxy esters previously reported and displayed greater or same potency than the best earlier prodrug form of this ligand. The combination of significant plasma stability and high potency suggests that these compounds would be appropriate for in vivo studies.

## Poster #6

| Mary Schad | ***Design and synthesis of insulin sensitizers for the treatment of Type II Diabetes*** |
|---|---|

Background: Type II diabetes (T2D) is a chronic disease characterized by impaired insulin secretion, insulin resistance, and hyperglycemia. T2D affects nearly half a billion people globally. Current treatments seeking to improve insulin sensitization have been largely ineffective. To address this unmet need, research in the Kerns lab is exploring the development of insulin-sensitizing compounds targeting the GLUT4 pathway. GLUT4 is a glucose transporter that resides in the cytosol of fat and muscle cells until insulin triggers its translocation to the cell surface where it facilitates glucose uptake. The GLUT4 pathway is negatively impacted by insulin resistance, and thus presents an attractive therapeutic target for treating T2D.

Objectives: Previous work yielded lead compound C59, that increases GLUT4 translocation in the presence of insulin, and identification of the protein target UNC119.(1) It is my aim to design and synthesize derivatives of C59 to generate new lead compounds with enhanced potency and improved drug-like properties. The immediate goal of this work is to identify new, superior derivatives of C59 for preclinical evaluation in murine models of T2D.

Methods: Using the crystal structure of UNC119 bound to C59 along with in silico ADMET prediction models, I will design and synthesize new derivatives, introducing hydrophilic and hydrophobic groups to strengthen compound-target interactions. Each derivative will be evaluated for its effect on insulin-mediated GLUT4 translocation, and the results from these assays will further guide the design of next-generation lead compounds.

Results: To date, I report the design and synthesis of seven novel analogs of C59 that are currently being tested for effect on insulin-mediated GLUT4 translocation in cell-based models.

Conclusion: Guided by testing results from current compounds, and from ongoing compound design using in silico models, effort continues to focus on synthesis of new derivatives to optimize potency and drug-like biophysical properties.

1. Yin, T.; Van Vranken, J.G.; Srivastava, D.; Mittal, A.; Buscaglia, P.; Moore, A.E.; Verdinez, J.A.; Graham, A.; Walsh, S.A.; Acevedo, M.A.; Kerns, R.J.; Artemyev, N.O.; Gygi, S.P.; Sebag, J.A. Insulin Sensitization by Small Molecules Enhancing GLUT4 translocation. Cell Chemical Biology 2023, 30(8):933-942 PMID: 37453421 DOI: 10.1016/j.chembiol.2023.06.012.

## Poster #7

| **Pornpoj "Jay" Phruttiwanichakun** | ***Local delivery of CST6 mRNA for bone regeneration*** |
|---|---|

Background: The treatment of large bone defects often calls for reconstructive surgeries, especially those involving bone grafts. However, bone grafting procedures are accompanied by the risks of graft rejection and donor site morbidity. Biodegradable implants, in combination with osteogenic agents, present a potential alternative to conventional bone defect treatment.

Objectives: To assess whether recombinant human CST6 (rhCST6) and rhCST6-encoding chemically modified mRNA (CST6-cmRNA) can promote osteogenesis and/or bone matrix mineralization in vitro and in vivo.

Methods: In vitro osteogenic studies were performed by treating MC3T3-E1 (subclone 4) murine pre-osteoblasts and human bone marrow-derived mesenchymal stem cells (hBMSCs) with various doses of rhCST6 protein in osteogenic media. End-point assessments for osteogenicity were alkaline phosphate activity (day 7) and alizarin red staining (day 21). hCST6-encoding mRNA (m1Ψ (1.0), m5C (1.0)) was generated using in vitro transcription, and formulated as lipid nanoparticles (LNPs) using rapid microfluidic mixing. The LNPs were tested for cytotoxicity and transfection efficiency in MC3T3-E1 and hBMSCs using MTS assay and ELISA, respectively. Finally, for an in vivo study, CST6-LNP (25-µg mRNA equivalence) as well as other control and test formulations were lyophilized onto an adsorbable collagen type I sponge for use as an implant in a rat metaphyseal bilateral drill defect model. The treatment efficacy (new bone growth) was assessed based on micro-CT and histological data.

Results: Firstly, rhCST6 protein (100 ng/mL) significantly increased alkaline phosphatase activity in hBMSCs after seven days of treatment in osteogenic media ($p < 0.05$). rhCST6 protein (0.01 to 1 ng/mL) also led to dose-dependent mineralization in MC3T3- E1 based on alizarin red staining. Cap1 CST6-cmRNA (m1Ψ (1.0), m5C (1.0)) with an A120 tail encapsulated in LNPs showed minimal cytotoxicity in RAW264.7, MC3T3-E1, and hBMSCs (MTS assay). Expression of rhCST6 from transfected MC3T3-E1 and hBMSCs was quantified in the supernatant using ELISA, yielding between 6 to 17 ng/mL of secreted rhCST6. Finally, for an in vivo study, CST6-LNP (25-µg mRNA equivalence) as well as other control and test formulations were lyophilized onto an adsorbable collagen type I sponge for use as an implant in a rat metaphyseal bilateral drill defect model. Using micro-CT, histology, and RT-qPCR for osteogenic and osteoclastic markers, the CST6-LNP group showed no significant differences compared to the lyophilized excipient control (LA) in all measurements on week 6 after implantation. In contrast, the lyophilized rhCST6 protein (15-µg protein) group showed a significant increase in relative bone volume in the defect area ($p < 0.05$) compared to the LA group.

Conclusion: In summary, rhCST6 protein was shown to promote osteogenic differentiation and mineralization in vitro, and bone regeneration in vivo. However, its CST6-LNP counterpart failed to promote bone regeneration in vivo, signifying the need for further investigation and optimization of the LNP formulation, the mRNA dose, and the method of LNP delivery.

## Poster #8

| **Paras Gaur** | ***Observing PARP1 interactions with G-quadruplexes and PARylation at single-molecule level*** |
|---|---|

Human genome contains numerous repetitive nucleotide sequences that display a propensity to fold into non-canonical DNA structures including G-quadruplexes (G4s). These unique structures have both positive and negative impacts on various aspects of nucleic acids metabolism including preservation of genome stability, DNA replication, DNA repair and RNA transcription. Numerous proteins have been discovered to interact with and modulate G4s. Poly (ADP-ribose) polymerase (PARP1), a DNA damage sensing enzyme, has been recently shown to bind G4s. PARP1 is an important anticancer drug target, especially in cancers deficient in DNA repair by homologous recombination, or displaying so-called BRCAness. Multiple PARP inhibitors including Olaparib, Rucaparib, and Niraparib have been approved by FDA for treatment of BRCA-deficient breast and ovarian cancers. Activity of these compounds was extensively studied with respect to PARP1 interaction with and its enzymatic activity on the canonical PARP1 substrates, namely single-strand and double-strand breaks (SSBs and DSBs). PARP1 has been reported to interact with a subset of G4s and to undergo auto-PARylation. The mechanism of this interaction, however, is poorly understood. Utilizing single-molecule total internal reflection fluorescence microscopy (smTIRF), we demonstrate that PARP1 dynamically interacts with G4s. Mass Photometry data indicate that a single PARP1 molecule is sufficient to catalyze auto-PARylation on a G4 substrate resulting in the addition of poly (ADP-ribose) chains with molecular weight of several hundred kDa. PARylation is more robust on the G4 DNA than on the SSB. PARP inhibitors exhibit a spectrum of PARP1 trapping ability, with EB-47 demonstrating the strongest effect, followed by decreasing potency with olaparib and veliparib. We utilized Mass photometry to study them, and it confirms that all three inhibitors prevent PARylation of G4-bound PARP1 without disrupting the PARP1-G4 interaction itself.

## Poster #9

| Yan Xu | ***In Situ Immunization with a TLR9 Agonist Virus-Like Particle to Activate Immune Response Against Leukoplakia Progression*** |
|---|---|

Background: Leukoplakia is the most common premalignancy in the oral cavity, characterized by white patches or spots forming inside the mouth. Risk factors include chronic irritation from smoking, alcohol consumption, and chewing betel nuts. The majority of oral squamous cell carcinomas (OSCCs) arise from oral leukoplakia. However, leukoplakia is insufficiently researched, making both diagnosis and treatment problematic. Diagnosis is highly subjective, and treatments (e.g., watchful waiting, conservative drug therapies, and surgical removal) have limited efficacy. There are no specific biomarkers for leukoplakia, and diagnosis heavily depends on the pathologist's analysis of biopsies, which can introduce significant variances. Therefore, further understanding and effective therapy for leukoplakia are highly desirable to reduce the risk of recurrence and malignant transformation.

Objectives: This study focuses on the immune signature of leukoplakia and explores whether stimulating the immune system with the immune adjuvant vidutolimod can prevent the progression of oral leukoplakia or even cause disease regression.

Methods: Human OSCC and leukoplakia samples were studied histologically and immunologically to reveal the local immune profile. A traditional murine model for inducing oral cancer was used to study leukoplakia development. Vidutolimod, an immune adjuvant (a bacteriophage Qβ protein shell encapsulating a Toll-like receptor (TLR)-9 agonist (G10)), was administered with anti-PD1, an immune checkpoint inhibitor, to achieve maximal immune response against leukoplakia. The treatment effect was analyzed histologically and immunologically. The immune profile was revealed by analyzing immune cell populations in oral lesions, draining lymph nodes, and cytokine changes in serum before and after treatment.

Results: From human leukoplakia analysis, elevated levels of CD11c+ and CD8+ T cells in severe leukoplakia suggested that the immune environment becomes more active as the disease progresses. The levels of pDC and Ki67+ CD8+ T cells in draining lymph nodes increased consistently under vidutolimod treatment combined with anti-PD1, indicating that the treatment successfully boosted the immune response. The therapeutic effect was also indirectly evidenced by decreased Ki67+ staining in vidutolimod and anti-PD1 treated mice. As a proliferation marker, higher Ki67+ cell expression is associated with poor prognosis. Therefore, the decrease in Ki67+ staining indicated the potency of combining vidutolimod and anti-PD1 against oral leukoplakia.

Conclusion: This study elucidated the dynamics of the immune response against leukoplakia progression under conditions likely to induce pDC activation combined with immune checkpoint blockade. It demonstrates the modulatory effects of immune therapies on both local and systemic immune responses. These findings reinforce the importance of strategically combining immune modulators like vidutolimod and checkpoint inhibitors in managing and potentially reversing high-risk precancerous conditions.

## Poster #10

| Cynthia Okafor | ***Exploring Bacterial Cellulose and Nanocellulose for Biomanufacturing*** |
|---|---|

Background: Cellulose, a complex carbohydrate and polysaccharide consisting of hundreds to thousands of β-linked D-glucose units, is widely used across various fields such as engineering, biomedicine, pharmaceuticals, and materials science. As the most abundant polysaccharide, cellulose is sourced sustainably from a variety of plants, several prokaryotes, and even one animal species, making it a key material for ongoing manufacturing needs. Its biodegradability, high crystallinity, and other versatile properties further enhance its potential applications, particularly in drug delivery systems and wound dressing.

Iowa, for instance, leads the USA in corn stover production, an agricultural byproduct that can be converted into cellulose. By leveraging this resource, alongside microbial cellulose sources, Iowa could become a leader in biomanufacturing, contributing to the growing demand for sustainable materials.

Objectives: This research focuses on maximizing agricultural and microbial sources for cellulose production, with an emphasis on engineering high-quality fibers suitable for applications like wound dressings.

Methods: The early stages of the study involve the production of bacterial cellulose (BC) from Kombucha SCOBY and nanocellulose from commercially sourced purified cellulose. A series of physicochemical characterizations such as XRD, BET, TGA/DSC, SEM, ATIR, etc., were performed to assess the quality of the produced cellulose to guide subsequent biofunctionalization and optimization processes.

Results: Initial results from X-ray diffraction (XRD) and scanning electron microscopy (SEM) on BC samples revealed unusual properties compared to those documented in the literature, prompting a refinement of the production process. This led to the introduction of a washing step to remove excess sugars from the BC. After process optimization, XRD confirmed the high crystallinity expected of cellulose, while SEM displayed the characteristic network of interwoven cellulose nanofibers. Thermogravimetric analysis (TGA) further demonstrated the thermal stability of the produced cellulose.

Conclusion: This study is a work in progress. The next phase of the research will focus on modifying the bacterial cellulose and nanocellulose, for use in wound dressings, drug delivery systems, and other biomanufacturing applications, ultimately aiming to meet various human needs in healthcare and industry.

## Poster #11

Eman Kamel

***Forced degradation of cell-based therapeutic products using various stresses and use of flow imaging microscopy for identification of damaged cells and cell debris.***

Background and objectives: Cell-based therapeutic products hold promise for treating various diseases, with cell lines like A20 B lymphoma serving as valuable models for studying the effects of stress conditions on cell therapies. This study aims to assess the effects of various stress conditions, including freeze-thaw stress, shaking stress, and thawing temperature, on A20 B lymphoma cell suspensions. The study focuses on utilizing FlowCAM 8000 as a novel technique to evaluate total cell concentration, cell viability, symmetry, and cell size distribution before and after exposure to stress conditions. Understanding these stress factors is essential for optimizing the formulation and storage of cell-based medicinal products to ensure their quality and efficacy.

Method: A20 B lymphoma cell suspensions were subjected to forced degradation studies mimicking conditions encountered during the production and handling of cell-based therapeutic products. Stress conditions included freezing with different DMSO concentrations, thawing at various temperatures, and shaking stress. Cell viability and cell counts were assessed using a hemocytometer and FlowCAM analysis after each stress condition.

Results: The study evaluated the effects of different concentrations of DMSO, Cell suspensions supplemented with DMSO at varying concentrations (0%, 1%, 2.5%, 5%, 10%, and 20% v/v) were subjected to one freeze-thaw cycle. Higher DMSO concentrations (5% and 10% v/v) showed better cell recovery post-thawing compared to lower concentrations (0% and 1% v/v).Cell counts significantly decreased with 0% and 1% DMSO, indicating a loss of cell viability under these conditions.

Shaking Stress: Shaking at 185 rpm for 3 hours resulted in increased cellular debris, correlated with reduced cell viability and total cell count. The presence of FBS in cell suspensions led to additional particles.

Thawing Temperature: Thawing at higher temperatures (37°C) yielded higher recovery of viable cells compared to lower temperatures. Flow imaging microscopy highlighted the impact of thawing temperature on cell quality and recovery.

Conclusion: This study demonstrates that thermal, freeze-thaw, and shaking stresses are critical factors to consider when formulating cell-based medicinal products. By employing a combination of analytical methods such as FlowCAM analysis, hemocytometry, and automated cell counting, it is possible to gain a comprehensive understanding of the effects of stress conditions on cell integrity and functionality. These findings underscore the importance of optimizing storage and handling protocols to maintain the quality of cell-based therapeutic products.

## Poster #12

| **Siddhanth Hejmady** | **A Comparative Study of Agitation-Induced Aggregation of Monoclonal Antibodies via Horizontal Shaking and Orbital Shaking** |
|---|---|

Background: Monoclonal antibodies (mAbs) have substantially improved treatments for diseases like cancer and rheumatoid arthritis. However, protein aggregation poses a major concern, compromising their stability, efficacy, and safety. Aggregation can arise from various stresses, including oxidative, thermal, and interfacial, which can lead to harmful immunogenic responses. Interfacial stresses are particularly challenging throughout the lifecycle of a drug, from manufacturing, transportation, storage, and clinical administration, where agitation, such as during shipping, can disrupt interfacial films of amphiphilic proteins formed via molecular adsorption to the interfaces and release aggregates into the solution (Eppler, 2010). To evaluate mAb stability against these stresses, the industry uses scale-down methods like shaking studies. However, there is no standard guideline for these methods, and variables such as shaking type, intensity, container type, and formulation characteristics can impact aggregation, particularly of subvisible particles and research on the influence of these parameters is limited (Morales, 2023).

Objectives: This study investigates how different shaking types—horizontal versus orbital—affect mAb aggregation, focusing on Cetuximab and Tocilizumab.

Methods: Both mAbs were extracted from commercial products, purified, and prepared in phosphate-buffered saline (PBS) at 10 mg/mL, then diluted to 0.5 mg/mL. Samples were subjected to shaking at 200 RPM and 25°C for 72 hours, and aggregation was assessed using a variety of analytics including flow imaging microscopy.

Results: Results showed that unagitated Cetuximab had 320 particles/mL, increasing to 403 particles/mL with orbital shaking and 28,150 particles/mL with horizontal shaking, indicating high sensitivity to horizontal shaking. Conversely, Tocilizumab showed minimal change, with unagitated samples at 217 particles/mL, increasing to 293 particles/mL with orbital shaking and decreasing to 213 particles/mL with horizontal shaking.

Conclusion: These findings highlight the need for appropriate shaking methods in stability assessments to ensure accurate evaluation of mAb formulations. Cetuximab's sensitivity to horizontal shaking suggests the importance of standardizing agitation conditions to develop robust mAb products.

References:
(1) Eppler A. Relevant shaking stress conditions for antibody preformulation development. European journal of pharmaceutics and biopharmaceutics. 2010.
(2) Morales AM. End-to-end approach to surfactant selection, risk mitigation, and control strategies for protein-based therapeutics. The AAPS journal. 2023.

## Poster #13

| Zoe Kramin | ***Development of Real-Time Predictions for Organic Slurry Composition and Polysaccharide Utilization Loci Metabolism*** |
|---|---|

Background: Greenhouse gas emissions are on the rise, prompting COP26 participants to pledge significant changes to mitigate the increase in average atmospheric temperature. In 2021, the United States and European Union introduced the Global Methane Pledge, with over 100 countries committing to reduce methane emissions by 30% by 2030. Because the methane emissions from decaying organic waste in landfills and the agricultural sector are substantial, there is a critical need to develop advanced conversion technologies to process heterogeneous organic wastes. Increasing biomethane production from the billions of tons of annual food and agricultural waste through anaerobic digestion (AD) is a recognized solution to the energy and climate crisis. However, challenges such as digester instability due to variable organic feedstock composition must be addressed. Additionally, advancements are needed for complex organics, like polysaccharides, which may not be effectively degraded by typical AD microbiomes.

Objectives: This study: 1) used diffuse reflectance spectroscopy (DRS) and machine learning to produce accurate and real-time predictions of feedstock slurry and digestate compositions, 2) used the predictions to evaluate digester stability over time, and 3) will investigate Bacteroidetes, a phylum prevalent in the human colon microbiome, that uses polysaccharide utilization loci (PUL) to degrade various carbohydrates through the production of various CAZymes.

Methods: This study used online DRS to analyze digester influent and effluent samples at the Muscatine Water Resource Recovery Facility. The data set used to train the supervised machine learning model contained spectral data and measured values for various constituents, including volatile fatty acids (VFAs) and alkalinity. Spectral data preprocessing included second derivative transformation and standard scaling to enhance signal quality. The model calibration was evaluated using the coefficient of determination (R2) for each chemical parameter. After calibration, the model was validated, and the root mean squared error (RMSE) was calculated to assess prediction accuracy. Digester stability was assessed by calculating the VFAs to alkalinity ratio, with values exceeding the critical threshold of 0.3 indicating process instability.

Results: The prediction accuracy for the 14 chemical parameters tested varied, with R2 values ranging from 0.23 to 0.99. The key indicators, VFAs and alkalinity, were accurately predicted with $R^2$ of 0.92 and 0.99, respectively, and RMSE of 0.06 and 0.89 g L-1, respectively. Stability was monitored in real time with predicted VFA and alkalinity values, achieving an RMSE of 0.019, with all predicted and reference values remaining below the instability threshold of 0.3.

Conclusion: This study demonstrated that DRS and machine learning can provide accurate, real-time monitoring of chemical parameters critical to the AD process.

Future Work: We hypothesize that DRS and machine learning can be used to predict kinetic hydrolysis rates for AD slurries containing complex substrates, including polysaccharides. Furthermore, we hypothesize that co-digestion of complex substrates will lead to the highest increase of the relative abundance of PUL-associated taxa, compared to mono-digestion and easily degraded polysaccharides. Models made with DRS may be able to predict the relative abundance of PUL-associated taxa as an indirect association with the metabolic fingerprints of this decomposition pathway.

## Poster #14

**Mortezaali Razzaghi**

***CRYO-EM STRUCTURAL STUDIES OF HUMAN RAD52 DNA REPAIR PROTEIN***


Mortezaali Razzaghi[1], Nicholas J. Schnicker[2,3], Maria Spies[1]

[1] Department of Biochemistry and Molecular Biology, University of Iowa Carver College of Medicine, 51 Newton Road, Iowa City, IA 52242, USA. maria-spies@uiowa.edu

[2] Protein and Crystallography Facility, University of Iowa, Iowa City, IA 52242, USA.

[3] Department of Molecular Physiology and Biophysics, University of Iowa Carver College of Medicine, 51 Newton Road, Iowa City, IA 52242, USA



Human RAD52 is a DNA and RNA binding protein that plays crucial roles in DNA repair and maintenance of genome stability. Depletion of RAD52 is synthetically lethal with defects in genome caretakers BRCA1 or BRCA2, which is observed in a quarter of breast and ovarian cancers. This makes RAD52 an attractive target for therapy in BRCA-deficient cancers. RAD52 consists of 418 amino acids, which forms an undecameric ring structure. Its N-terminal domain (1-212) is responsible for self-oligomerization and DNA binding activity while the C-terminal domain (213-418) is structurally disordered protein-protein interacting region. RAD52 has several functions at the stalled damaged replication forks. Little is known about the structural mechanisms of RAD52 interactions with the replication fork and partner proteins. In this study, we determined the structure of the undecameric ring of human RAD52 in the absence and presence of fork DNA using cryo-electron microscopy (cryo-EM). The apo form of the human RAD52 ring is very similar to the structure of the RAD52 N-terminal domain determined by crystallography and cryo-EM. RAD52 forms head-to-head arrangement of two undecameric rings in the presence of fork DNA based on mass photometry and cryo-EM data.


**References**


- Divya S. Bhat, M. Ashley Spies, Maria Spies. A moving target for drug discovery: Structure activity relationship and many genome (de)stabilizing functions of the RAD52 protein. DNA Repair 120 (2022) 103421

## Poster #15

| Ramkrishna Sen | ***Co-loaded Nanoparticle Formulation of Encorafenib and Trametinib for Targeting BRAF V600E in Colon Cancer*** |
|---|---|

Background: The combination of a BRAF V600E small molecule inhibitor (e.g., encorafenib or dabrafenib) and a MEK small molecule inhibitor (e.g., trametinib or binimetinib) are now options as second line therapy for metastatic CRC (mCRC) patients possessing BRAF V600E, providing a more effective blockade of the MAPK/ERK pathway than either drug alone. Poor water solubility, poor bioavailability, nonspecific distribution, and the development of resistance make it challenging for its emergence as a primary therapy. Thus, there is a need to improve the efficacy of the BRAF V600E targeting such that dual targeting RAS-RAF-MAPK pathway can accommodate further additions to the therapeutic regime without affecting safety. In this study we investigated the development and subsequent administration of a nanoparticle formulation co-loaded with the encorafenib and trametinib at a clinically relevant ratio and delivered at doses equating to maximum tolerated clinical doses in a murine xenograft CRC (HT-29: BRAF V600E) model.

Objectives:

- To develop a nanoparticle formulation co-loaded with encorafenib and trametinib at a clinically relevant ratio.
- To enhance the treatment efficacy of BRAF V600E metastatic colorectal cancer (mCRC) by targeting the RAS-RAF-MAPK pathway.
- To improve the drug solubility, bioavailability, and reduce resistance compared to current therapies.
- To evaluate the therapeutic impact of the nanoparticle formulation in a murine xenograft CRC model (HT-29: BRAF V600E) at maximum tolerated clinical doses.

Methods: We developed PLGA-based nanoparticles (ET-NPs) co-loaded with encorafenib and trametinib using nanoprecipitation for CRC treatment. NPs were characterized by DLS, TEM and tested for anticancer activity in HT-29 cells. Cellular uptake was assessed via confocal microscopy and flow cytometry. IVIS imaging confirmed tumor localization in vivo. In a murine xenograft model, HT-29 cells were injected into nude mice. Once tumors reached ~200 $mm^3$, mice were treated with DPBS (control), free ET (oral), or ET-NPs (IV), following a dosing schedule on days 0, 3, 7, and 11.

Results: By encapsulating encorafenib and trametinib within PLGA NPs, we aimed to enhance their solubility, stability, and bioavailability while minimizing systemic toxicity. The ET-NPs had an average size about of 41.30 ± 3.07 nm, smooth surface morphology, optimal drug loading (E: 89.56 ± 6.77 µg/mg, T: 0.656 ± 0.24 µg/mg). Cellular uptake study confirmed time-dependent uptake by HT-29 cells. In vivo tumor localization and biodistribution of DiR-labelled ET-NPs in in vivo was confirmed by IVIS imaging system. Upon formulation of ET in to PEGylated polymeric NPs, tumor uptake of ET-NPs was significantly improved compared to free drug, and this was reflected in the improved the overall antitumor efficacy of ET-NPs in vivo.

Conclusions: This study identified that the combination of the BRAF inhibitor encorafenib and the MEK inhibitor trametinib demonstrated superior anti-tumor activity in a HT-29 (BRAF V600E) xenograft model, both in vitro and in vivo, compared to the soluble drug form. These findings could lead to more effective therapies for CRC patients, improving outcomes and quality of life. This combination is currently not used in patient treatment; thus, it deserves an opportunity to be included in clinical trials.

## Poster #16

| Apurva Dusane | *Nanomedicine for Fuchs endothelial corneal dystrophy* |
| --- | --- |

Background: Fuchs endothelial corneal dystrophy (FECD) is a polygenic disorder characterized by thickening and softening of Descemet membrane (DM), guttae formation (extracellular collagenous deposits in a mound shape on DM), loss of corneal endothelial cells (CECs), and edema of the corneal stromal layer, along with that result in vision loss of vision.

Purpose: Currently, surgical procedures are the only first line treatment for Fuchs endothelial corneal dystrophy (FECD). We aimed to develop a nanomedicine approach for targeting corneal endothelial cells (CECs) to prevent FECD progression. Potent drug comprising nanoparticulate eye drops were used.

Methods: Nanoparticles were characterized by measuring size and charge using Zetasizer. Drug loading and encapsulation efficiency were quantified using HPLC-DAD. Particles were characterized using XRD. Formulation stability was examined for 3 months at 4°C and 25°C. Release studies were performed at 37°C. Safety and Efficacy of formulation was evaluated on mice with a well-characterized FECD mutation (129S6/SvEvTac Col8a2Q455K). Optical coherence tomography (OCT) and confocal imaging were performed to study progression of guttae and changes in CEC morphology. An initial baseline for these techniques on a mouse cohort was recorded at the age of 4 months, then subsequently after 1 month of therapy (age of 5 months) and after 2 months of therapy (age of 6 months).

Results: Particle size was determined to be 96.69±1.69 nm with a narrow polydispersity index (PDI) (n = 4 batches). XRD indicated drug incorporation in nanoparticles. Formulation exhibited in vitro controlled drug release. In the mouse model, the nanoparticulate drug therapy had a good safety profile and low off-target side-effects. CCM indicated an overall improvement in cell health with nanoparticle treatment.

Conclusions: Findings indicate the successful development of drug-loaded targeted nanoparticles as a possible drug delivery strategy for CECs to prevent FECD progression.

## Poster #17

| Sophie Granger | ***Human hnRNPA1 reorganizes telomere-bound Replication Protein A*** |
|---|---|

Background: Human replication protein A (RPA) is a heterotrimeric ssDNA binding protein responsible for various aspects of cellular DNA metabolism. The dynamic interactions of the four RPA DNA binding domains (DBDs) with DNA control replacement of RPA by downstream proteins in various cellular metabolic pathways. RPA plays several important functions at telomeres where it binds to and melts telomeric G-quadruplexes, non-canonical DNA structures formed at the G-rich telomeric ssDNA overhangs. Understanding its dynamic interactions, particularly at telomeres, is critical for elucidating its role in DNA protection.

Objectives: This research aims to investigate how heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) remodels RPA when bound to telomeric ssDNA and to explore the implications of this remodeling on telomere protection.

Methods: We employed single-molecule total internal reflection fluorescence microscopy (smTIRFM) and mass photometry (MP), complemented by biophysical and biochemical analyses, to study the interactions between RPA, telomeric ssDNA, and hnRNPA1.

Results: It was previously thought that hnRNPA1 displaces RPA off the DNA, however, our findings reveal that hnRNPA1 specifically remodels RPA bound to telomeric ssDNA by dampening the RPA configurational dynamics and forming a ternary complex. Uniquely, among hnRNPA1 target RNAs, telomeric repeat-containing RNA (TERRA) is selectively capable of releasing hnRNPA1 from the RPA-telomeric DNA complex.

Conclusion: The telomere-specific RPA-DNA-hnRNPA1 complex we identified may play a crucial role in telomere protection, linking our findings back to the broader implications for cellular DNA metabolism and the stability of telomeres.

## Poster #18

| Rebecca Splitt | ***Investigating E-cadherin Mediated Force Transmission*** |
|---|---|

Background: Cells experience both external and internal forces throughout their lifetimes. To withstand these forces, cells trigger energetically costly rearrangements of the actin cytoskeleton and growth adhesion complexes. This process is initiated when Epithelial cadherin (E-cadherin) senses force and signaling is propagated by the recruitment and activation of a master regulator of metabolism, AMP-activated protein kinase (AMPK). This kinase stimulates a signal transduction cascade that results in reinforcement of the actin cytoskeleton and ATP production to pay for the energetic costs of reinforcement. There are still many questions that remain on how cells respond to force. For example, most of the studies in the literature only examine a narrow range of amplitudes of force, while cells experience a wide range of amplitudes of physiological forces.

Objective: This study investigates how cells respond to different amplitudes of shear stress. I hypothesize that unique activators phosphorylate AMPK at low and high amplitudes of shear stress.

Methods: Orbital shear stress will be applied to MCF10a cells. Following the application of force either immunofluorescence or western blot will be used to analyze samples. Commercially available inhibitors and shRNAs targeting the AMPK activator CAMKK�� will be used prior to the application of force.

Result: Inhibiting CAMKK��disrupts AMPK activation and cytoskeletal reinforcement in response to force.

Conclusion: Cells response to force varies with increasing amplitudes of force. These findings begin to extend the paradigm of how cells sense and respond to force and provide how diseases associated with defective force transmission and metabolism arise.

## Poster #19

| Hesham Refaat | ***Challenges with Removal and Addition of Polysorbates From/To Formulations of Monoclonal Antibodies*** |
|---|---|

Background: Formulating therapeutic proteins, such as monoclonal antibodies (mAbs), requires precise control over excipients, particularly surfactants like polysorbates, which ensure protein stability. Small-scale techniques, often employed in research labs, are less understood compared to large-scale manufacturing processes. The removal or addition of polysorbates in these formulations is a key challenge, especially for smaller research facilities, which lack the sophisticated tools available to larger pharmaceutical companies. This study investigates the effectiveness of membrane separation techniques for surfactant removal and buffer exchange, aiming to provide a solution for the accumulation of polysorbates during protein isolation.

Objectives: This research aims to compare two methods of polysorbate removal in mAb formulations: the use of centrifugal concentrators and a surfactant removal column, followed by buffer exchange. Additionally, it assesses the impact of polysorbate reintroduction on the stability of trastuzumab (Tmab), a therapeutic mAb, after buffer exchange, with the goal of optimizing excipient management in small-scale protein formulation.

Methods: Trastuzumab (Tmab) was processed using two methods to remove and reintroduce polysorbates. The first approach used a 30 kDa centrifugal concentrator membrane for buffer exchange. The second approach employed a surfactant removal column designed for efficient polysorbate extraction, followed by buffer exchange. The concentration of polysorbate 20 was measured before and after the processes using a fluorescence assay. The stability of Tmab was assessed through various techniques, including Size Exclusion Chromatography (SEC), Dynamic Light Scattering (DLS), Flow imaging microscopy, Circular Dichroism (CD), and fluorescence spectroscopy.

Results: The surfactant removal column was found to completely eliminate polysorbates from the formulation, while the centrifugal concentrator method only reduced the polysorbate concentration slightly. Subsequent buffer exchanges with the addition of polysorbate resulted in significant polysorbate accumulation, particularly in formulations where the excipient was present during buffer exchange. This accumulation affected the protein's secondary structure, as observed through CD analysis, indicating a structural disruption. However, other methods like SEC, DLS, and UV-2nd derivative fluorescence showed no significant impact on protein aggregation or overall stability.

Conclusion: Centrifugal concentrators alone are insufficient for complete polysorbate removal, leading to accumulation in Tmab formulations when excipients are present during buffer exchange. The two-step approach, where polysorbate is added after buffer exchange, provides a more controlled and stable formulation. These findings highlight the importance of carefully managing excipients in therapeutic protein formulations, particularly for smaller research facilities lacking large-scale manufacturing capabilities. This approach could improve protein stability during formulation and ensure the consistency of biotherapeutics, especially in early-stage pharmaceutical development.

## Poster #20

| Maclaine Putney | ***Diffuse reflectance spectroscopy for non-destructive composition measurements of algal biofilm*** |
|---|---|

Present standard practice for algae compositional analysis requires samples be collected from the production system and analyzed offline. These offline laboratory tests are not only time consuming they also require highly trained personal. The destructive nature of present compositional analysis also presents economic loss to growers since potentially marketable biomass is removed for testing. Diffuse reflectance spectroscopy has been shown in other industries to be highly reliable at analyzing chemical properties of commercial products for quality assurance purposes. This research aims to prove that diffuse reflectance spectroscopy can be used to measure compositional parameters of algal biofilms such as biomass growth and lipid content faster and non-destructively. With the use of a small sensor (The Texas Instruments DLP NIRscan) absorbance spectra was collected from an experimental lap scale rotating algae belt (RAB) system. The spectra included the wavelengths between 900 and 1700 nm and were collected before the biomass was harvested to simulate inline measurement sampling. A total of 4 scans were taken per one singe composite biomass sample and averaged to create an absorbance spectra training file. The biomass was then analyzed in the lab to find the volatile solids content and the total lipid content. Total volatile solids can be used as a surrogate for total biomass productivity a key parameter of successful algae production systems. Machine learning was used on the training files that were created with the averaged absorbance spectra and resulting lab data. A partial least squares (PLS) regression model was used to compare the true values with the model's predicted outputs. The results showed a strong correlation between the volatile solids and the measured absorbance spectra (R2=0.97). This strong correlation can be associated with the definite NIR signature captured by our sensor in the1300-2400 nm range, which is the absorbance bands that most affect water content in plants. The lipid content data also showed a fair correlation (R2=0.73), this weaker correlation is most likely due to the interference of the water content of the sample since there is an overlap in the characteristic spectral range for lipids and water between 1350 and 1500 nm.

## Poster #21

| **Samuel Yu** | ***Akt inhibition: potential therapeutic target for PI3K-driven sarcomas?*** |
| --- | --- |

Background: Sarcomas are a heterogenous group of cancers with few effective targeted therapies. PI3K signaling is frequently activated in sarcomas due to loss of PTEN in 30-60% of samples, which represents a common therapeutic target. PI3K signaling regulates the transcriptional co-activators TAZ and YAP to drive a transcriptome that promotes tumor growth in sarcomas. This PI3K-TAZ/YAP axis works in parallel to the more well-defined PI3K-Akt-mTORC1 axis and together promotes tumor growth and survival, thus providing a rationale for combination therapy. Dual inhibition of TAZ/YAP-TEAD and mTORC1 synergistically reduced proliferation and anchorage independent growth in cell lines and showed a synergistic effect in a xenograft mouse model. However, tumors remained after the treatment and may be partly attributed to incomplete targeting of the PI3K pathway. Previous studies have shown that mTORC1 inhibition leads to feedback activation of Akt in various cancer cell lines. Akt signaling promotes survival by phosphorylating several downstream substrates including the FOXO transcription factors and BCL-2 family proteins which regulate apoptosis.

Objectives: The objective is to determine whether targeting Akt, upstream of mTORC1, can further abrogate oncogenic PI3K signaling in sarcomas and reduce tumor growth. Additionally, we would like to target TAZ/YAP-TEAD and Akt in combination to see if targeting higher up in the PI3K signaling pathway shows better efficacy than the combination of mTORC1 and TAZ/YAP-TEAD inhibition.

Methods: The sarcoma cell lines A204 (malignant extrarenal rhabdoid tumor) and RH30 (alveolar rhabdomyosarcoma) will be used in our experiments. To determine whether Akt signaling promotes sarcoma growth and survival, we will utilize pharmacological inhibitors of Akt to assess how Akt inhibition affects proliferation, clonogenic outgrowth, anchorage-independent growth and phosphorylation of downstream substrates. Akt inhibitors will also be used in combination with TAZ/YAP-TEAD inhibitors to determine if targeting both pathways further decreases tumor growth and survival.

Results: Targeting Akt with the inhibitors MK2206 or Capivasertib reduced proliferation of A204 and RH30 cells at concentrations of 1μM and higher when treated for 72 hours. By western blot, phosphorylation of substrates downstream of the Akt-mTORC1 pathway were also decreased at concentrations of 1μM and more so at 10μM. Clonogenic outgrowth also followed a similar trend in both A204 and RH30. Combination treatment with either MK2206 or Capivasertib and the TEAD inhibitor VT-107 further decreased proliferation in both cell lines.

Conclusion: While initial data shows that targeting Akt and TAZ/YAP-TEAD may likely have a more additive effect on proliferation and clonogenic outgrowth, there may be a difference on anchorage-independent growth which needs to be determined. More experiments will be performed to determine how inhibition of Akt affects these hallmarks of cancer and how the combination therapy affects apoptotic signaling proteins downstream of Akt such as FOXO transcription factors and BCL-2 family proteins such as BAD. Upon completion of these experiments, we will also perform xenograft study to assess how tumor growth is affected by combination therapy in vivo.

## Poster #22

| Hossein Zare | *In Vitro Dispersion of Pseudomonas aeruginosa Biofilms* |
| --- | --- |

Background: Bacterial Biofilms are made up of bacteria held together by a self-secreted extracellular polymeric substance. Pathological bacterial biofilms are responsible for 60% of all chronic bacterial infections. They are resistant to current treatment strategies due to mechanisms of antibiotic resistance specific to biofilm. Current treatments to fight against nosocomial infections (infected implants) typically involve the removal of the infected implant, extensive antibiotic therapy, and re-implantation, which is invasive and costly. Thermal shock has emerged as a promising method to reduce bacterial populations on medical implants. However, the underlying mechanisms remain unclear. Bacterial reduction may occur due to in-situ death within the biofilm or via the dispersion of live cells responding to the stress of elevated temperatures. Dispersed bacteria resulting from thermal shock pose a potential risk of entering a patient's bloodstream. Hence, quantifying the extent to which bacteria disperse into surrounding tissues and the bloodstream under thermal shock conditions is critically important.

Objectives: Our research aims to characterize the effect of thermal shock on biofilm dispersion In Vitro. In this regard, we investigated the bacterial dispersal rate within an In Vitro turkey meat model.

Hypothesis: We hypothesize that there is a dynamic equilibrium between biofilm and planktonic states, and that thermal shock affects planktonic and biofilm bacteria differently. We also hypothesize that biofilm bacterial transport follows Fickian model.

Methods: In static dispersion experiments, Pseudomonas aeruginosa biofilms were submerged in media (phosphate buffered saline or tryptic soy broth) and monitored for bacterial detachment and re-adhesion. Additionally, a flow cell setup was used to minimize reattachment, where planktonic bacteria were flushed out at flow rate of 40 mL/min. For the turkey meat model, biofilms were cultured for 24 hours at 37°C and then covered with 350 µm thick slices of turkey meat. These slices were exposed to varying time scales and analyzed to quantify bacterial penetration into the meat layers.

Results: In static experiments, we observed that biofilm dispersion reaches equilibrium quickly, maintaining a stable minimum bacterial population (log 6.7 CFU/$cm^2$), regardless of initial biofilm population or media volume. In the flow cell experiments, a similar minimum population was recorded (log 6.9 CFU/$cm^2$). Thermal susceptibility tests revealed that planktonic bacteria are rapidly reduced under thermal shock (70-80°C) within 1 minute, while biofilm populations required more than 15 minutes of exposure for significant reduction. In the turkey meat model, we observed bacterial penetration into the meat layers following the diffusion model equation. Dispersion rates were calculated based on the diffusion equation, with values on average close to 2*10$^{-}$10 $m^2$/s. Moreover, dispersal rate calculated based on the second Fick's law in a diaphragm cell is reported to be 7.5 *10$^{-}$10 $m^2$/s.

Conclusion: Bacterial dispersion transport seems to follow Fickian model but with a dispersal rate as high as it suggests an active mode of bacterial movement. This results in the rapid equilibrium observed between biofilm and surrounding media which increases the thermal demands for biofilm mitigation.

## Poster #23

| Souradip Sinha | *The ATAC HAT complex is a critical epigenetic complex driving sarcomagenesis* |
|---|---|

Background: Sarcomas are diverse neoplasms that originate in mesenchymal tissues and most sarcomas currently lack effective therapies. Our lab has previously shown that the Hippo signalling pathway is perturbed in sarcomas, and TAZ/YAP, the end effectors of the Hippo pathway, are key oncoproteins driving sarcomagenesis. In the vascular sarcoma Epithelioid hemangioendothelioma (EHE), the predominant oncogenic driver is a fusion protein TAZ-CAMTA1, which is produced by a genetic translocation, where the N-terminal end of WWTR1 (gene name for TAZ) is fused in-frame with C-terminal domains of CAMTA1, which is another transcription factor.

In EHE, TAZ-CAMTA1 promotes cancer hallmarks by interacting with YEATS2 and ZZZ3, which are key scaffolding subunits of the epigenetic complex called the Ada2A-Containing (ATAC) complex. The ATAC complex has a histone acetyltransferase (HAT) module containing GCN5/PCAF which are HAT enzymes and are known to promote gene transcription by acetylating H3K9.

Objectives: We are studying the role of the ATAC complex as an oncogenic driver in both fusion protein-positive and -negative sarcomas. In EHE, TAZ-CAMTA1 drives cancer hallmarks in vitro and in vivo. Hence, we are investigating whether genetic/pharmacological inhibition of the ATAC complex can decrease the transformed phenotype. Similarly, we are investigating what are the cancer hallmarks that are driven by the ATAC complex in other sarcomas.

Methods: To study the ATAC complex, we are genetically knocking down ATAC subunits. For siRNA-mediated, cells with si-YEATS2/ZZZ3 are compared with si-non targeting (si-NT). For shRNA-mediated knockdown, cells with sh-YEATS2/ZZZ3 were compared with sh-empty vector(shEV) and sh-NT. For the cell-line derived xenograft experiment, we injected NSG mice with SW872(TAZ-CAMTA1) cells with sh-NT (negative control) or sh-YEATS2#1 or sh-YEATS2#3. For the in vitro drug studies, sarcoma cells were treated with varying drug concentrations and compared with vehicle control (DMSO).

Results: siRNA-mediated knockdown of YEATS2 in SW872(TAZ-CAMTA1) cells decrease H3K9-acetyl levels. We also observed that YEATS2 knockdown significantly reduced tumor progression in vivo as compared to non-targeting cells.

Previously, data from The Cancer Genome Atlas showed that high YEATS2/ZZZ3 RNA levels corelated with worse overall survival in sarcomas. We have observed that YEATS2/ZZZ3 protein levels are also significantly upregulated in human sarcoma cell lines as compared to their negative controls. In the SKLMS cells, we have observed that YEATS2 knockdown significantly decreases H3K9-acetyl levels, reduces 2D proliferation, and anchorage-independent growth (soft-agar plates).
We have also observed via western blot analysis that the treatment of sarcoma cells with PU139 (pan-HAT inhibitor) or GSK4027 (GCN5/PCAF-specific) significantly decreases H3K9-acetyl levels.

Conclusion: In sarcomas, the ATAC complex is a major acetyltransferase complex required for the maintenance of H3K9-acetylation levels, which is a critical histone post-translational modification necessary for transcriptional activation. In the context of EHE, we show that YEATS2 is necessary for tumor progression in vivo, which suggests that TAZ-CAMTA1 drives cancer hallmarks in an ATAC complex-dependent manner in EHE. Our data also show that in SKLMS, the ATAC complex acetylates H3K9, promotes 2D proliferation and anchorage-independent growth. Hence, the ATAC complex is emerging as a critical oncogenic epigenetic complex, that could be therapeutically targeted in sarcomas.

## Poster #24

| ***Adam Benmoussa*** | ***The Crystallization and Structure of Human Alternative Replication Protein A*** |
|---|---|

Background: Essential for all aspects of cellular DNA metabolism, replication protein A (RPA) is the primary eukaryotic single-strand DNA (ssDNA) binding protein. Composed of 3 subunit proteins, RPA1, RPA2, & RPA3 each contain DNA-binding domains (DBDs). RPA subunits oligomerize at DBDs-CDE, a site referred to as the trimerization core. In primates, an alternative version of RPA can coexist with RPA called alternative RPA (Alt-RPA). Alt-RPA is composed of subunits RPA1, RPA4, and RPA3 (DBDs-CGE), a trimerization core differing from RPA. RPA and alt-RPA both bind to ssDNA with similar affinity, resulting in RPA and alt-RPA competing over ssDNA.

Objectives: We hypothesize due to the structural differences between RPA and alt-RPA's trimerization cores, the binding specificity exhibited by small-molecule DIDS-like inhibitors indicates DIDS-like inhibitors are binding to RPA and alt-RPA trimerization cores. Our aims include solving the structure of alternative RPA and locating the DIDS binding site on RPA and alt-RPA.

Methods: Using Förster Resonance Energy Transfer (FRET) based assays, we have measured RPA and alt-RPA bound to ssDNA, quantifying RPA and alt-RPA inhibition. FRET-based analysis has identified small molecule DIDS-like inhibitors that exhibit a degree of specificity inhibiting canonical and alternative human RPA at different concentrations. The current structure of full-length alt-RPA is unknown. Until this point, no experimentally solved structure containing RPA4 existed. I have crystallized the partial trimerization core of alt-RPA (RPA4-3) in conditions with and without DIDS to experimentally solve RPA4's structure and identify specific inhibitory binding sites on alt-RPA in the future. Molecular replacement was utilized to solve RPA4-3's structure using an RPA2-3 structure.

Results: Structural analysis of DBD-G has identified F135 within DBD-D is replaced with P134 in DBD-G. In DBD-D, F135 is one variation of two nucleotide base-stacking aromatic residues conserved across all RPA DBDs.

Conclusion: Analysis of x-ray diffraction data will identify binding site location(s) while biophysical FRET-based analysis determines inhibition stoichiometry and specificity. Alt-RPA promotes CAG-trinucleotide repeat expansion, the cause of Huntington's disease. Solving alt-RPA's structure, investigating alt-RPA ssDNA secondary structure melting, and identifying inhibitors specific to alt-RPA are the beginning steps towards developing a pharmaceutical treatment for Huntington's Disease.

## Poster #25

| Mohammad Alnatour | ***Active Targeting of Colorectal Cancer Using Chemotherapy-loaded Nanoparticles Functionalized with Folate Receptor-α (FRα) Ligand, Pemetrexed*** |
|---|---|

Background: Colorectal cancer (CRC) is the third most frequent cause of cancer deaths in the United States and more than 50,000 patients die from CRC each year. These alarming statistics highlight the inadequacies of current treatment modalities (e.g., chemotherapy and radiotherapy) particularly for advanced CRC. Chemotherapeutics that comprise the first and second-line treatments for advanced CRC have substantial systemic side effects that limit treatment effectiveness. This underscores the pressing need for innovative therapeutic strategies that selectively target neoplastic cells while sparing healthy tissues. Tumor-targeting nanoparticles (TTNPs) present a viable solution to these challenges. TTNPs are capable of delivering therapeutic agents directly to neoplastic cells, thereby augmenting treatment efficacy and mitigating systemic toxicity. To achieve this objective, biocompatible polymers were fabricated de novo, linked to a novel targeting moiety, pemetrexed, a folic acid receptor-α (FRα) ligand. FRα is known to be overexpressed by 34% of human CRCs while not being expressed by most healthy tissues. The overexpression FRα in aggressive CRC makes it an appealing target for tumor-specific drug delivery. The current research landscape is primarily focused on the utilization of folic acid as a targeting moiety, however, pemetrexed has a higher affinity for FRα and has never been used before as a targeting ligand. This project aims to evaluate the ability of pemetrexed conjugated polymers to generate TTNPs loaded with chemotherapeutic agents and test them in a murine FRα+ CRC model.

Methods: Pemetrexed was employed to synthesize tumor-targeting PLGA-PEG-pemetrexed polymers via a ring opening polymerization (ROP) reaction of lactide and glycolide. The resultant tumor-targeting polymer was used to produce TTNPs. The targeting efficiency of TTNPs loaded with coumarin 6 (C6) was evaluated using CT26 cells, a FRα-expressing CRC cell line, through cellular uptake studies, employing flow cytometry and confocal microscopy. The ability of TTNPs to accumulate in FRα-expressing tumors was assessed in CT26 tumor-bearing BALB/C mice using In Vivo Imaging System (IVIS) imaging. The anticancer activity and safety profile of paclitaxel (PTX)-loaded TTNPs were evaluated by in vivo antitumor efficacy testing and body weight measurements, respectively. CT26 tumor-bearing BALB/C mice were dosed with PTX-loaded TTNPs, Taxol (positive control), empty TTNPs (negative control) and non-targeting formulations (NTNPs) via IV tail injection equivalent to 10 mg/kg of PTX, two doses one week apart.

Results: Pemetrexed terminated PLGA-PEG copolymer was successfully synthesized by ROP of lactide and glycolide using PEG-pemetrexed as an initiator in the presence of the catalyst, Sn(Oct)2. The polymer structure was validated by NMR spectroscopy. The calculated molecular weight of the polymer was ~20 kDa and lactide:glycolide ratio was 50:50. The tumor targeting polymer was used to synthesize PTX-loaded TTNPs using a single emulsion method. TTNPs had a hydrodynamic diameter of 160 nm and a Zeta potential of -21 mV. Flow cytometry experiments showed that the uptake of TTNPs by CT26 cells was significantly higher compared to NTNPs made with commercially available PEG-PLGA.

# Thank you for attending this year's CBB Conference!

To help us improve our future conferences, we ask that you take few moments to fill out this post-conference survey.

https://forms.office.com/r/aGm5G3vBR3