2025
August 1st

VANDERBILT INSTITUTE *of* CHEMICAL BIOLOGY
STUDENT RESEARCH SYMPOSIUM
VANDERBILT STUDENT LIFE CENTER

MORNING SESSION	
8:30 am - 8:35 am	**Welcome Remarks: Doug Mitchell, PhD** (Director, Vanderbilt Institute of Chemical Biology)
Oral Session I *Armstrong Prize Winner, Faculty/Trainee Tag Team, & Trainee Flash Talks* (Xavier Streety, Chair)	
8:35 am – 9:05 am	**TRAINEE FLASH TALKS** Raleigh Jonscher (Fiona Harrison lab), Christina Jimenez (Jens Meiler lab), Scott Benner (Jeff Johnston lab), Mudit Agarwal (Jens Meilier lab), Kateryna Nabukhotna (Borden Lacy lab), Jake Hermanson (Lars Plate lab)
9:05 am – 9:25 am	**RICHARD ARMSTRONG PRIZE WINNER** **Ryan Fansler** (Wenhan Zhu lab) *NO Country for B. theta: A Novel sRNA-DNA-Transcription Factor Complex Coordinates Commensal Nitrosative Stress Response*
9:25 am – 9:55 am	**John Wilson, PhD** (Assoc. Prof. of Chemical & Biomolecular Engineering, VU)**/ Jake Schulman** *Engineering Polymeric STING Agonist Delivery Platforms for Cancer Immunotherapy*
9:55 am – 10:00 am	Break
Oral Session II *Deerfield, St. Jude, VU Faculty/Trainee Tag Team & Trainee Flash Talks* (Alexis Pope, Chair)	
10:00 am-10:30 am	**TRAINEE FLASH TALKS** Valentina Guidi (Daria Kim lab), Shravan Dommaraju (Doug Mitchell lab), Preston Gourville (Jeff Johnston lab), Maowei Hu (Daniel Blair lab, St. Jude), Ashraf Mohammed (Aseem Ansari lab, St. Jude), Kate Clowes Moster (Chuck Sanders lab)
10:30 am– 10:50 am	**Nadim Shohdy, PhD** (Chief Operating Officer, 3DC) *Deerfield Management: Translating research from bench to market*
10:50 am -11:20 am	**Allison Walker, PhD** (Assistant. Prof of Chem, VU) **/ Emilee Patterson** *Prediction of elicitor regulator pairs to enable secondary metabolite discovery*
11:20 am – 11:50 pm	**Daniel Blair, PhD** (Assistant Member, St. Jude) *Fragmentation-First Experimentation*
LUNCH BREAK 12:15 pm – 1:15 pm	
11:55 am – 12:55 pm	**Q & A with Dr. Burke** (RSVP-only event)
AFTERNOON SESSION	
Oral Session III *Faculty/Trainee Tag Team & Trainee Flash Talks* (Will Sutton, Chair)	
1:00 pm – 1:30 pm	**Brian Bachmann, PhD** (Stevenson Prof. of Chem., VU)**/Kathryn Penton** *Lost on the cutting room floor, spirocyclic β-lactone polyketides editing spliceosome function*
1:30 pm – 1:55 pm	**TRAINEE FLASH TALKS** Mayuresh Gadgil (Doug Mitchell lab), Clay Tydings (Jens Meiler lab), Mason Wilkinson (Chuck Sanders lab), Sophia Hamza (Jens Meilier lab), Harrison Parent (Colleen Niswender lab)
1:55 pm – 2:25 pm	**Kevin Schey, PhD** (Prof of Biochem, VU)**/ Sarah Zelle** *Proteomic analysis of adult Nrf2 and αB-crystallin mutant zebrafish lenses under oxidative stress*
2:25 pm - 2:30 pm	Break
Oral Session IV *Keynote* (Molly Sullivan, Chair)	
2:30 pm - 3:30 pm	**Martin Burke, PhD** (May and Ving Lee Professor for Chemical Innovation & Professor of Chemistry, University of Illinois Urbana-Champaign) *Imagine a world where anyone can make molecules*
RECEPTION	
3:30 pm – 5:00 pm	Reception & Poster Session

VANDERBILT UNIVERSITY
School *of* Medicine Basic Sciences
Vanderbilt Institute of Chemical Biology

Imagine a World Where Anyone Can Make Molecules

Martin Burke, PhD
May and Ving Lee Professor for Chemical Innovation & Professor of Chemistry, University of Illinois at Urbana-Champaign

Marty Burke is the May and Ving Lee Professor for Chemical Innovation at the University of Illinois at Urbana-Champaign. He is the founding Director of the Molecular Maker Lab and a co-founder of the Molecule Maker Lab Institute. He also helped launch the Carle Illinois College of Medicine and served as its inaugural Associate Dean of Research. He received his undergraduate degree in chemistry from Johns Hopkins University, an MD from Harvard Medical School and the Massachusetts Institute of Technology, and a PhD from Harvard University.

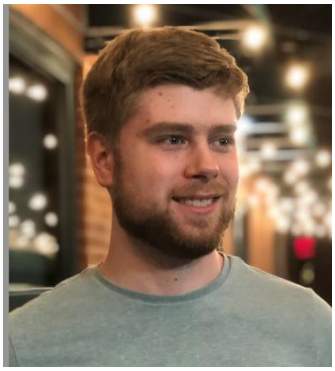
Marty developed block chemistry, which is chemistry that anyone can do. His lab specifically pioneered the modular synthesis of small molecules with MIDA/TIDA boronate building blocks, an approach that is friendly to automation, non-specialists, and AI. More than 300 of these building blocks are now commercially available, and they have been used by hundreds of other labs worldwide to help identify many different types of natural products, pharmaceuticals, herbicides, pesticides, fungicides, diagnostic probes, catalysts, anti-corrosive coatings, quantum dots, carbohydrate sensors, and a wide range of materials, collectively yielding >1000 publications including >300 patents. In his own lab, Marty leveraged this block chemistry approach to develop the field of molecular prosthetics yielding new drug candidates for cystic fibrosis (now in clinical trials) and anemia, define the sterol sponge mechanism by which glycosylated polyene macrolide natural products kill eukaryotic cells which led to renal sparing antifungal candidates for treating invasive fungal infections (now in clinical trials), and to enable AI-guided closed-loop discovery of top-in-class organic lasers and mechanistic insights underlying the stability of organophotovoltaic materials. Through the Molecule Maker Lab, Marty is working with partners from many different backgrounds to democratize molecular innovation. Leveraging the broad potential of this block chemistry approach, Marty (co)-founded multiple biotechnology companies, including REVOLUTION Medicines, Sfunga Therapeutics (now Elion Therapeutics), cystetic Medicines, and Excelsior Sciences, which have collectively advanced seven drug candidates into clinical trials.

Marty is an elected member of the National Academy of Medicine and American Society for Clinical Investigation, and a Fellow of the American Association for the Advancement of Science. He is also a winner of the ACS Cope Scholar Award, ACS Elias J. Corey Award in Organic Synthesis, Hirata Gold Medal, Mukaiyama Award, Presidential Medallion from the University of Illinois, and ACS Nobel Laureate Signature Award for Graduate Education in Chemistry. He has also been recognized many times as a Teacher Ranked as Excellent by the University of Illinois.

1st August 2025 | **RICHARD ARMSTRONG PRIZE FOR RESEARCH EXCELLENCE**

NO Country for B. theta: A Novel sRNA-DNA-Transcription Factor Complex Coordinates Commensal Nitrosative Stress Response

Ryan Fansler: *Wenhan Zhu Research Group, Vanderbilt University*



Ryan Fansler is a fifth-year Ph.D. candidate in the Microbe-Host Interactions Program at Vanderbilt University. He earned his undergraduate degree from Vanderbilt in 2020, majoring in Biochemistry and Chemical Biology, and then entered graduate school through the Interdisciplinary Graduate Program. In 2021, he joined the lab of Dr. Wenhan Zhu. Ryan's research focuses on how the commensal gut microbiota adapts and persists during episodes of intestinal inflammation. His thesis work explores how Bacteroides thetaiotaomicron, a prominent gut commensal, employs a small RNA-mediated defense against nitrosative stress to remain resilient in the inflamed gut.

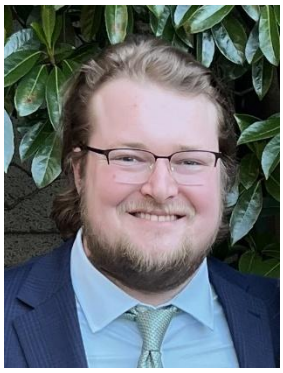
1st August 2025 | **TAG TEAM SPEAKERS**

Engineering Polymeric STING Agonist Delivery Platforms for Cancer Immunotherapy

John Wilson, PhD: *Associate Professor of Chemical & Biomolecular Engineering, Vanderbilt University*

John T. Wilson is an Associate Professor of Chemical & Biomolecular Engineering and Chancellor Faculty Fellow at Vanderbilt University, where he also Co-Leads the Host-Tumor Interactions Program within the Vanderbilt-Ingram Cancer Center. Dr. Wilson graduated from Oregon State University with a B.S. in Bioengineering and received his Ph.D. in Bioengineering from the Georgia Institute of Technology. He was awarded a Cancer Research Institute (CRI) Postdoctoral Fellowship to support his postdoctoral research at the University of Washington. Dr. Wilson started his independent career at Vanderbilt in 2014 and was promoted to Associate Professor with tenure in 2021. His multidisciplinary Immunoengineering Laboratory works at the interface of engineering and immunology to improve human health, bringing together expertise in molecular engineering, nanotechnology, and pharmaceutical science to develop strategies for more precisely modulating immune and inflammatory responses at the tissue, cell, and subcellular level. He has published more than 50 scientific articles, including in high-impact journals such as Nature Nanotechnology, Science Immunology, and Nature Biomedical Engineering. He is a Fellow of the American Institute for Medical and Biological Engineering (AIMBE) and has received several awards including the NSF CAREER award, Alex's Lemonade Stand Foundation 'A' Award, Stand Up To Cancer Innovative Research Grant Award, CBME Young Innovator Award, and Vanderbilt Chancellor's Award for Research.

Jake Schulman: *John Wilson Research Group, Vanderbilt University*



Jacob (Jake) A. Schulman is a 3rd year Ph.D. candidate in the Biomedical Engineering program at Vanderbilt University under the direction of Dr. John T. Wilson. He received his B.S. in Biomedical Engineering from North Carolina State University and the University of North Carolina at Chapel Hill (Lampe Joint Department of Biomedical Engineering). As an undergraduate, Jake worked in Dr. David Zaharoff's Immunoengineering Lab to develop polymeric cancer immunotherapy and hapten-conjugate vaccine platforms. He also worked at Lindy Biosciences, a biologic formulation company, as a Research and Development Engineer. At Vanderbilt University, Jake has earned a National Science Foundation graduate research fellowship and works at the intersection of cancer immunology and polymer chemistry to improve the safety profile and antitumor efficacy of STING agonists. Outside of the lab, Jake enjoys watching football, going on hikes, and spending time with his fiancée, Molly.

Prediction of elicitor regulator pairs to enable secondary metabolite discovery

Allison Walker, PhD: *Assistant Professor of Chemistry, Vanderbilt University*

Allison Walker, Ph.D. completed her Bachelor's degree in chemistry at Brown University, working in the research lab of Professor Sarah Delaney. She then completed her PhD at Yale University in Professor Alanna Schepartz's lab. Allison then completed a postdoc at Harvard Medical School in Professor Jon Clardy's lab before starting her independent career. She is currently an Assistant Professor of Chemistry and Biological Sciences at Vanderbilt University. Her lab's research focuses on developing AI and other computational methods for natural product discovery, engineering of biosynthetic pathways, and design of peptide therapeutics.

Emilee Patterson: *Allison Walker Research Group, Vanderbilt University*

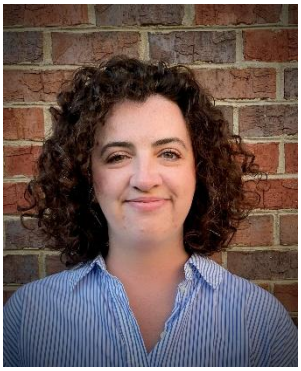
Emilee is a PhD candidate in Allison Walker's Lab at Vanderbilt University, where she studies regulation of biosynthetic gene clusters in Streptomyces, focusing on the transcription factor JadR2. She began her research career at Hartwick College conducting molecular studies with Dr. Eric Cooper. She then worked as a laboratory technician at Albany Medical College under Dr. Carlos de Noronha, studying host-pathogen interactions. At Vanderbilt, she was a research assistant in Dr. Rafael Arrojo e Drigo's lab, investigating metabolism in diabetic mice. Emilee has mentored students through the START and FGLI programs and serves as Vice President of the Graduate Student Association.

Lost on the cutting room floor, spirocyclic β-lactone polyketides editing spliceosome function

Brian Bachmann, PhD: *Stevenson Professor of Chemistry, Vanderbilt University*

Brian O. Bachmann is Stevenson Endowed Chair. Professor of Chemistry in the Vanderbilt University Departments of Chemistry and Biochemistry. As principal investigator of the Vanderbilt Laboratory for Biosynthetic Studies, his research is concerned with biosynthesis and discovery of natural and unnatural products, and their application to understanding and manipulating biological systems ranging from microbial ecologies to human biology. Prior to Vanderbilt, Brian was Director of Chemistry at Ecopia Bioscience, where he helped build the first 'genome mining' program for natural product discovery. Brian completed his doctoral studies in Chemistry at The Johns Hopkins University in the laboratory of Professor Craig A. Townsend, where he discovered and characterized beta-lactam synthetase, and his Masters in Science at Southern Methodist University, working with Professor John D. Buynak in chemical synthesis of mechanism based inhibitors of beta-lactamase. Brian's interest in biosynthesis was sparked by an impactful undergraduate research experience in the laboratory of Tomas Hudlicky on the biocatalytically enabled synthesis of inositols.

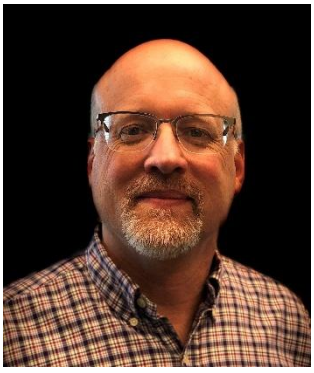
Kathryn Penton: *Brian Bachmann Research Group, Vanderbilt University*



Kathryn Penton earned her Bachelor's Degree in Chemistry in 2016 from Delta State University in Cleveland, MS where she stayed to earn her Master's of Science in Natural Sciences in 2018. Throughout her time at Delta State, Kathryn worked in Dr. Sharon Hamilton's laboratory developing profile libraries of drug-loaded natural polymer fiber mats for wound healing uses. Kathryn moved to Nashville, TN in 2018 and is a Ph.D candidate in the Department of Chemistry working in the laboratory of Dr. Brian Bachmann. Her research has focused on natural product drug discovery and leveraging knowledge of pharmacophore biosynthesis to identify and isolate a retinue of oxazolomycin family compounds. Work has revealed structure-activity relationships pertaining to cytotoxicity in acute myeloid leukemia and the unreported activity of this compound class to selectively modulate alternative splicing. As aberrant RNA splicing occurs in nearly all cancer types, the oxazolomycins represent an unexplored opportunity for therapeutic development.

Proteomic analysis of adult Nrf2 and αB-crystallin mutant zebrafish lenses under oxidative stress

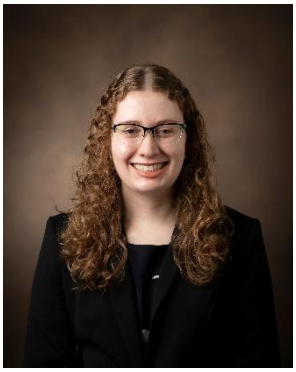
Kevin Schey, PhD: *Professor of Biochemistry, Vanderbilt University*



Dr. Schey received his B.S. in Chemistry from Muhlenberg College in Allentown, PA and his Ph.D. in Analytical Chemistry from Purdue University working under Dr. Graham Cooks. After post-doctoral training at the University of Chicago he began his academic career at the Medical University of South Carolina in 1990. In 2008 he moved to the Mass Spectrometry Research Center (MSRC) and Department of Biochemistry at Vanderbilt University. He has secondary appointments in the Departments of Chemistry and Ophthalmology and Visual Sciences. His research interests lie in the areas of lens biochemistry, proteomics of aging, exosome proteomics, and imaging mass spectrometry. He directs MSRC Core laboratories in Imaging MS, Proteomics, and small molecule MS. He has published over 200 papers

and has been elected Fellow of the American Association for the Advancement of Science and of the Association for Research in Vision and Ophthalmology.

Sarah Zelle: *Kevin Schey Research Group, Vanderbilt University*



Sarah is a 6th year PhD candidate in the Chemical and Physical Biology Program. She received dual BS degrees in biochemistry and genetics from Iowa State University in 2020. While at Iowa State, she studied the membrane determinates of SynGAP in the lab of Dr. Eric Underbakke. She came to Vanderbilt through IGP in 2020 and joined the labs of Dr. Hassane Mchaourab and Dr. Kevin Schey. Her dissertation project uses zebrafish and mass spectrometry proteomics to better understand age-related cataract formation.

1st August 2025 | **INDUSTRY SPEAKERS**

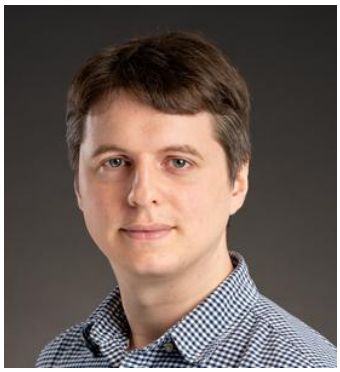
Translating research from bench to market

Nadim Shohdy, PhD: *Chief Operating Officer, 3DC, Deerfield Management*

Nadim Shohdy, Ph.D., is the Chief Operating Officer, Deerfield Discovery and Development and joined the Firm in 2024. Prior to Deerfield, Dr. Shohdy was Vice President, Corporate Development at Hyku Biosciences. Before Hyku Biosciences, Dr. Shohdy served as Entrepreneur in Residence at RA Ventures, a division of RA Capital Management, during which time he also served as Vice President, Corporate Development at Clear Creek Bio. Prior to these roles, Dr. Shohdy spent 10 years at NYU Langone Health in roles of increasing seniority, including most recently as Associate Dean, Therapeutics Alliances. Dr. Shohdy started his career as a Research Associate at Cold Spring Harbor Laboratory before serving as a Research Analyst, Innovation Fund at the International AIDS Vaccine Initiative. Dr. Shohdy holds a B.S. in Biology from Stony Brook University and a M.A., M.Phil and Ph.D. in Microbiology from Columbia University.

Fragmentation-First Experimentation

Daniel Blair, PhD: *Assistant Member, St. Jude Children's Research Hospital*



Dr. Daniel Blair completed both his MSci and PhD with Varinder Aggarwal at the University of Bristol, UK, working on stereospecific organolithium and organoboron chemistry. This was followed by a postdoc at University of Illinois at Urbana-Champaign with Martin D Burke which centered on automated chemical synthesis, and was supported by a Damon-Runyon Fellowship. In August 2022, he became a part of the St. Jude faculty, as an Assistant Member in the Department of Chemical Biology & Therapeutics. His lab focusses on developing high-throughput technologies for creating and comparing small molecules in biological contexts.

SESSION I.

L-Ascorbic Acid Enhances Catecholamine Synthesis in Differentiated SH-SY5Y Cells.

Raleigh Jonscher – *Fiona Harrison Research Group, Vanderbilt University*

L-ascorbic acid (ASC, vitamin C) is a redox-active micronutrient that functions as both a cofactor for monooxygenases and a regulator of oxidative tone. Subclinical ASC depletion affects an estimated 10–30% of the U.S. population and may disrupt neurotransmission by impairing catecholamine biosynthesis and elevating oxidative stress. In rodent models, ASC deficiency reduces cortical and striatal dopamine (DA) and its metabolites, correlating with behavioral phenotypes indicative of dopaminergic dysfunction. Clinically, ASC supplementation demonstrates antidepressant efficacy and potentiates selective serotonin and norepinephrine reuptake inhibitors. We hypothesized that ASC enhances catecholamine synthesis through transcriptional regulation of biosynthetic enzymes. To evaluate this, we employed SH-SY5Y neuroblastoma cells, differentiated with 10 µM all-trans-retinoic acid for 7 days, as a model of human catecholaminergic neurons. This phenotype was validated with RNA sequencing, revealing expression of catecholamine biosynthetic enzymes and transporters. Following 24-hour exposure to 0, 50, 100, 200, or 500 µM ASC, intracellular monoamines and their metabolites were quantified via high-performance liquid chromatography. ASC induced a concentration-dependent increase in DA and norepinephrine along with elevated levels of 3-methoxytyramine and dihydroxyphenylacetic acid, indicating enhanced synthesis. Quantitative PCR revealed ASC-dependent upregulation of the key synthetic enzymes' phenylalanine hydroxylase and tyrosine hydroxylase, but not of catabolic genes including monoamine oxidase A, aldehyde dehydrogenase 2, and catechol-O-methyltransferase. These data support ASC enhancement of catecholamine biosynthesis via transcriptional activation of rate-limiting synthetic enzymes, rather than suppression of degradation pathways. This mechanistic insight provides a cellular basis for neurochemical effects of ASC and informs its potential utility as an adjunctive agent in disorders of catecholaminergic dysregulation.

Evaluating the structural basis of NSCLC mutant dimerization within the trans- and juxtamembrane region of EGFR and HER2

Christina Jimenez, Kaitlyn Ledwitch – *Jens Meiler Research Group, Vanderbilt University*

The trans- and juxtamembrane domains (TMD/JMD) of human epidermal growth factor receptors (HER/EGFR/ErbB) regulate the helix-helix dimer interface to control receptor tyrosine kinase (RTK) activation and inactivation. Patient-identified non-small cell lung cancer (NSCLC) mutations occur all along the TMD and JMD regions of EGFR and HER2. While these HER TMD/JMD-localized mutations are prevalent in cancer, our structural understanding of how these patient-derived TMD/JMD mutations hijack the normal oligomerization states of HER family proteins is lacking. Here, we use nuclear magnetic resonance (NMR) spectroscopy to assess 1) how oncogenic mutations within the TMD/JMD perturb the architecture/interactions of the dimer interface at an atomic level and to probe 2) residue-specific structural changes between TMD/JMD homodimer and heterodimer mutants in combinations with other HER family members. In this work, we showcase our preliminary NMR data for evaluating the structural changes for mutant TMD homodimers (i.e. EGFRA647T and HER2V659E) and heterodimers (i.e. EGFRA647T/HER2 and EGFR/HER2V659E). Our preliminary data show that these mutations perturb chemical shifts compared to wild-type protein indicative of TMD/JMD structural rearrangements. This experimental NMR framework is used to compute structural models for each mutant and their respective homo- and heterodimeric states. The NMR data presented here is part of a multidisciplinary approach and will be integrated with functional assays, fluorescence spectroscopy, and computational modeling to construct holistic models of HER activation mechanisms for selected NSCLC TMD/JMD variants.

Development of RyR2-Selective Small Molecules as Antiarrhythmic Agents

Scott Benner, Aaron Gochman, Tri Do, Daniel Blackwell, Christian Egly, Bjorn Knollmann – *Jeffrey Johnston Research Group, Vanderbilt University*

Cardiovascular disease is the leading cause of death in the United States, with approximately 659,000 occurring each year. Heart disease covers a broad spectrum of conditions, many of which involve cardiac arrhythmias. Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a potentially life-threatening genetic arrhythmia syndrome caused by gain-of-function mutations in the cardiac calcium channel (RyR2). One standard form of CPVT treatment is flecainide, a class 1c antiarrhythmic drug that blocks RyR2 in addition to cardiac sodium and potassium channels. Our findings, through collaboration with the Knollmann lab, suggest RyR2 inhibition as flecainide's principal mechanism of action in CPVT treatment and emphasize RyR2 as a significant therapeutic target. Although hyperactive RyR2 has been linked to a variety of heart diseases, there are currently no approved drugs that are RyR2-selective. Furthermore, off-target block of cardiac sodium and potassium channels can cause lethal ventricular arrhythmias and is a major safety concern. Using a hypothesis-driven approach, we aim to develop RyR2-selective small molecule antiarrhythmic agents that are not inhibitors of key off-target receptors, such as the sodium channel. Through a combination of structure-guided design and computational modeling, we synthesized flecainide-derived analogs that abrogated sodium channel block while leaving RyR2 inhibition unchanged. Preliminary data from whole cell patch-clamp assays supports our hypothesis that structurally modifying flecainide is a viable option for abolishing sodium and potassium channel activity. A selective RyR2-inhibitor would not only provide a framework for future therapeutic development but would improve the scientific community's understanding of dysregulated RyR2's role in heart disease.

In Silico Design of Prefusion-Stabilizing Mutations in Alphavirus Envelope Glycoproteins

Mudit Agarwal – *Jens Meiler Research Group*, *Vanderbilt University*

Alphaviruses encompass a genus of enveloped, positive-sense RNA viruses that comprise encephalitic (e.g., Venezuelan equine encephalitis virus, VEEV) and arthritogenic (e.g., Mayaro Virus, MAYV) pathogens. With no approved vaccines or antivirals for VEEV and MAYV, host immunity relies almost entirely on neutralizing antibodies directed against the prefusion conformation of the E1/E2 glycoprotein. Stabilizing the prefusion state prevents the irreversible burial of critical antigenic epitopes, thereby enhancing prefusion stability, solubility, and recombinant protein expression. To achieve such stabilization, a comprehensive in silico pipeline was implemented to engineer point mutations that can stabilize the prefusion architecture for both VEEV and MAYV envelope proteins. Initial structural models of the prefusion monomers were generated using AlphaFold2 and further refined using Rosetta Cartesian relax simulations. Comprehensive silico mutagenesis was subsequently employed, utilizing ESM, ProteinMPNN, MIF-ST, and ThermoMPNN to target residues within critical inter-subunit interfaces. Candidates then underwent rigorous energetic screening via Rosetta Cartesian DDG ($\Delta\Delta G < -2.24$ REU), surface-exposure analysis, and structural filtering to exclude mutations predicted to disrupt antigenic epitopes or essential folding networks. For VEEV, computational screening identified A94D and A717R as promising mutations for intradomain stabilization, while also recognizing S650F and S755T as the top-ranked prefusion-stabilizing substitutions based on energy. Structural analysis demonstrated that A94D and A717R introduce stabilizing hydrogen bonds between the E1 and E2 subunits, while S650F and S755T enhance packing interactions within the interface. Similar stabilizing mutations were identified at corresponding sites within the MAYV E1/E2 complex, suggesting a conserved stabilization mechanism across alphavirus envelope proteins. This computational approach provided a concise, experimentally testable set of prefusion-stabilizing mutations for alphavirus glycoproteins, offering a clear roadmap for subsequent experimental validation. We will present further explanation in the context of each step of this analytical pipeline.

Nanobodies against *Clostridioides difficile* CDTb provide a toolkit for toxin neutralization and quantitation

Kateryna Nabukhotna, David M. Anderson, Maria McGresham, John A. Shupe, Ruben Cano Rodriguez, Rebecca A. Shrem, Brian Wadzinski, Benjamin W. Spiller – *Borden Lacy Research Group, Vanderbilt University*

Clostridioides difficile is a pathogenic bacterium and a leading cause of antibiotic-associated life-threatening diarrhea in the United States. The symptoms of the infection arise due to production of toxin A and toxin B. These toxins disrupt the intestinal barrier and cause an acute host inflammatory response, a major hallmark of pathophysiology in C. difficile infection (CDI). Epidemic C. difficile strains additionally produce the C. difficile transferase toxin (CDT, a binary toxin consisting of catalytic CDTa and delivery CDTb) suggesting that it may be important for the severity of CDI. However, the role of CDT during C. difficile pathogenesis remains poorly understood, partially due to limited research tools for inhibiting toxin activity and knowing when the toxin is present. We created a CDTb-specific nanobody clone library and chose to express and purify five with promising CDTb-binding properties. Studies using the Carterra LSAXT platform revealed high affinity binding interactions with three distinct CDTb epitope groups. We tested the nanobodies from two different epitope bins as capture-detection pairs for their capacity to function in a sandwich ELISA assay. This assay enabled us to quantify CDTb produced in the cecum of mice over the time course of an in vivo infection. Additionally, we found that these nanobodies had potent neutralizing activities that protected epithelial Vero-GFP cells from CDT cytopathic effects. AlphaFold3 structure predictions allowed us to map neutralizing epitopes on the toxin to hypothesize mechanisms of toxin neutralization. We are working on developing in vitro functional assays to confirm predicted mechanisms by which nanobodies neutralize toxin activity, for example via blocking of CDTb oligomerization and assembly necessary for the delivery of catalytic CDTa into cell cytosol. We anticipate that these reagents will allow researchers to expand toxin intervention and monitoring strategies needed to obtain a deeper understanding of the CDT mechanism of action during CDI.

Public Cohort Analysis Identifies Thyroglobulin Variants as Hypothyroidism Risk Factors

Jake N. Hermanson, Andrew Hudson – *Lars Plate Research Group, Vanderbilt University*

Hypothyroidism, a condition characterized by deficient T3 and T4 hormone production, affects approximately 7% of Americans and is associated with symptoms such as impaired neurological development, metabolic dysfunction, depression, and weight gain. The disease arises from diverse causes, including abnormal thyroid development, autoimmune disorders, environmental exposures, and genetic mutations. Familial inheritance is frequently observed, but identifying specific genetic causes has been challenging due to the involvement of many genes and their high genetic variability. One such gene, thyroglobulin (Tg), encodes a 330 kDa precursor protein essential for T3/T4 synthesis and exhibits at least 21 known polymorphisms in the human population. Most hypothyroidism-linked mutations reported to date have been discovered through small-scale, targeted clinical studies. While informative, these studies lack the broader perspective provided by large-scale population data. In this study, we leverage the NIH All of Us research program to identify Tg mutations associated with hypothyroidism that we estimate affect over 150,000 people. We find that disease penetrance correlates with the efficiency of Tg protein secretion. Notably, many of these mutations remain uncharacterized, likely due to insufficient functional evidence supporting their pathogenicity. Here, we provide the first functional characterization of these Tg variants by examining their degradation pathways using nano-luciferase assays and evaluating their potential structural impacts through bioinformatic analyses. Identifying mutations that contribute to disease can enable earlier diagnosis and more effective prevention of hypothyroidism. These findings may also contribute to future efforts to better classify subtypes of hypothyroidism, which could inform more personalized treatment strategies. Overall, our study demonstrates that large-scale cohort analysis can uncover Tg mutations with meaningful clinical relevance and elucidates the molecular mechanisms by which they contribute to disease in the U.S. population.

SESSION II.

Accessing A Unique Class of Antibiotics: Total Synthesis of the Aminoglycoside Plazomicin
Valentina Guidi, Aaron Xu – *Daria Kim Research Group, Vanderbilt University*

Aminoglycosides are a class of potent, broad-spectrum antibiotic molecules used clinically for the treatment of severe bacterial infections. Plazomicin, a therapeutic agent against urinary tract infections, is of particular interest because unlike other aminoglycosides, it is resistant to most aminoglycoside-modifying enzymes, thus lowering its susceptibility to bacterial resistance. Plazomicin's structure consists of an unsaturated aminomonosaccharide residue, C4-methylated aminoarabinopyranoside residue, and 2-deoxystreptamine core. Traditionally, the synthesis of plazomicin has relied on the derivatization of the less potent natural isolate sisomicin, which highlights a major limitation in aminoglycoside synthesis: the difficulty in directly accessing structural modifications which instigate antibiotic resistance. We aim to circumvent this limitation by developing a total synthesis of plazomicin, allowing for a concise construction of the molecule via tailored modifications. We will target plazomicin's three core fragments separately and link the fragments via two glycosylation events. The 2-deoxystreptamine core will be synthesized from homoserine and involve a stereoselective fragment coupling and ring closing via a Henry reaction. The synthesis of the arabinose derivative will involve the key regioselective radical methylation of the C4 carbinol. Lastly, the unsaturated aminomonosaccharide residue will be accessed through a glutamic-acid-derived gamma-lactone and feature a C4-C5 olefin oxidation and cross-coupling to install the ethanolamine motif. Because most aminoglycosides are comprised of similar building blocks, the key steps of this plazomicin synthesis could be later utilized to access a variety of aminoglycosides.

Mining underutilized resources for hidden microbial metabolism
Shravan Dommaraju – *Douglas Mitchell Research Group, Vanderbilt University*

Microbial biosynthesis provides tremendous medicinal and biotechnological value. Access to genomic information for rare and understudied microbes has exploded, so tools for functional annotation and prediction are required to extract useful information. A staggering amount of public genomic data is deposited in short contigs and lacks annotation. We developed an advanced genome mining tool, MetaRODEO, to efficiently mine these unannotated and "low-quality" (meta)genomic data as a putative untapped biosynthetic reserve. We validated MetaRODEO by characterizing metabolites encoded by uncultured human-associated and coral-reef-associated microbes. Knowing that these "low-quality" data could lead to bona fide new metabolites, we targeted biosynthetic pathways from phylum Acidobacteria, a highly prevalent and biosynthetically rich taxon, which is difficult to culture. Through our efforts, we characterized biosynthesis of an Acidobacterial lasso peptide and multiple lipopeptides, representing some of the first unique natural products from this phylum. We further targeted extremophiles, which, owing to low culturability and habitat access, are inherently difficult to study. We first characterized a pathway from a hydrothermal vent metagenome, which produces a hypermodified, hyperanionic peptide bearing 14 phosphorylations. In addition, we examined a pathway from a hypersaline lake environment, which produces a peptide bearing up to 17 GABAylations. Biosynthesis of this "gabatide" repurposes eukaryotic tubulin-modifying enzymes to install a novel modification onto an unnatural prokaryotic substrate. These hyperanionic, hypermodified peptides are widespread throughout ecosystems and microbial taxa, suggesting they may play important evolutionary roles. Altogether, our efforts with MetaRODEO profiled an untapped source of public genomic data, resulting in a large dataset of novel biosynthetic pathways, the production of >10 new metabolites, and discovery of unprecedented biosynthetic enzymes.

A total synthesis of feglymycin enabled by a heterocyclic organocatalyst and Umpolung Amide Synthesis
Preston C. Gourville, Jade A. Bing, Rashanique D. Quarels, Sergey V. Tsukanov, Kenneth E. Schwieter, Kazuyuki Tokumaru, Amanda B. Stephens, Dawn M. Makley, Bo Shen, Abigail N. Smith – *Jeffrey Johnston Research Group, Vanderbilt University*

Aryl glycinamides are a common motif in natural products with antiviral and antibacterial properties. Strategies to access these non-canonical residues have relied on asymmetric methods such as the Sharpless aminohydroxylation of styrenes, and multistep synthesis targeting the desired amino acids that can be coupled using condensative coupling reagents. This approach presents several downsides, including the risk of epimerization of α-stereocenters, hazardous reagents, and the stoichiometric use of coupling reagents with high molecular weights. Shifting the paradigm of carboxylic acids and coupling reagents to Umpolung amide Synthesis (UmAS) brings together a halonitroalkane and N-halamine to forge amide bonds without epimerization risk. Furthermore, the non-condensative nature of UmAS requires a distinct complement of reagents with a less hazardous profile. However, UmAS has been untested on mid-sized peptides until now. This work will describe how asymmetric aza-Henry reactions with quinoline-based bis-amidine (BAM) organocatalysts can furnish the building blocks for the total synthesis of the tridecapeptide, antiviral natural product feglymycin.

Direct-to-Biology Enabled Molecular Glue Discovery
Maowei Hu – *Daniel Blair Research Group, St. Jude Children's Research Hospital*

Molecular glues powerfully control protein proximity but have largely eluded direct screening. A promising avenue for addressing this challenge lies within pinpointing the fundamental features for function-first identification of molecular gluing events. In the widely accepted mechanism, a molecular glue stabilizes two proteins within a ternary complex – here we show how differences in affinity for ternary and binary complexes directly categorize glues from non-glues. We leverage these differences together with high-throughput chemical synthesis and affinity-selection mass-spectrometry to discover a molecular glue degrader from a suite of over 20,000 crude chemical reaction mixtures. Orthogonal assays robustly support identification of molecular glues via ternary complex stability. Our findings suggest a roadmap for de novo molecular glue discovery lies within kinetic profiling of unpurified mixtures of small organic molecules against protein pairs.

Targeted Reactivation of Frataxin via Synthetic Genome Regulators: Mechanistic Insights into BET Bromodomain Engagement
Ashraf Mohammed – *Aseem Ansari Research Group, St. Jude Children's Research Hospital*

Friedreich's ataxia (FA) is a progressive neurodegenerative disease caused by transcriptional silencing of the FXN gene due to GAA trinucleotide repeat expansions. We developed synthetic genome readers/regulators (SynGRs), such as SynTEF1, which are bifunctional molecules composed of a sequence-specific DNA-binding polyamide tethered to a pan-BET ligand (e.g., JQ1). These constructs recruit BET family transcriptional coactivators to the silenced FXN locus to restore gene expression. To explore the mechanistic basis of BET engagement, we systematically evaluated SynGRs bearing bromodomain-selective ligands. Surprisingly, only SynGRs with pan-BET or BD2-selective ligands activated FXN transcription, while BD1-selective SynGRs were inactive in cells despite their high in vitro affinity for BD1. However, co-treatment with free BD1-selective ligands like GSK778 enhanced SynGR activity, revealing competitive BET protein dynamics between natural transcription factors and synthetic recruiters. Further structure-guided redesign of the chemical linker in BD1-selective SynGRs restored activity, pinpointing a critical spatial configuration necessary for productive chromatin engagement via BD1. These findings demonstrate that SynGRs mimic natural transcription factor behavior rather than functioning as chromatin mimics. Our work provides new insights into domain-selective BET recruitment and establishes a platform for the rational design of gene-targeted therapeutics.

High-Throughput Screening Identifies Small Molecule Modulators of KCNQ1 Trafficking
Katherine R. Clowes Moster, Mason C. Wilkinson, Carlos G. Vanoye, Katherine M. Stefanski, Kathryn R. Brewer, Alfred L. George Jr. – *Charles Sanders Research Group, Vanderbilt University*

Approximately 1 in 2500 individuals suffer from congenital long QT syndrome (LQTS), a cardiac disorder that can cause syncope, cardiac arrythmia, and cardiac arrest, which can be fatal. Loss-of-function mutations in the voltage gated potassium channel protein KCNQ1 cause type 1 long QT syndrome (LQT1), which accounts for 30-50% of cases of LQTS. Over 250 disease-associated mutations in KCNQ1 have been identified, but it is unknown whether there is a common mechanism through which these mutations impact KCNQ1. Previous studies have found that mistrafficking may play a role in KCNQ1 dysfunction. Only ~20% of expressed WT KCNQ1 successfully traffics to the plasma membrane, and many LQT1-associated mutations in KCNQ1 decrease trafficking efficiency further. Protein mistrafficking has been identified as a mechanism of several diseases and has been found to be rescuable with small molecules. This led us to hypothesize that mistrafficking is a common mechanism of KCNQ1 loss-of function in LQT1, and that small molecules can alter the trafficking efficiency of KCNQ1. To test this hypothesis, we developed a high content imaging-based trafficking assay to screen for compounds that alter the trafficking of KCNQ1 in mammalian cells. Screening of small molecule libraries identified several hits that alter KCNQ1 expression and/or trafficking. Specifically, one hit compound has been identified that increases cell surface KCNQ1 and leads to an increase in KCNQ1 current in CHO cells. An additional hit compound decreases total and cell surface KCNQ1. This compound has been shown to destabilize KCNQ1 and reduce the expression of disease-linked “super trafficking” KCNQ1 R231C mutant back to WT-like levels. Follow up experiments to determine the mechanism of action of these hits and begin preliminary SAR studies are ongoing. This work contributes to our larger hypothesis that misfolding-induced mistrafficking is a common, rescuable, mechanism of KCNQ1 dysfunction and informs potential routes for treatment of cardiac disorders like LQT1.

SESSION III.

Thio-oxazole modified peptides biosynthesized by multinuclear iron-dependent oxidative enzymes (MNIOs)
Mayuresh G. Gadgil, Shravan R. Dommaraju, Xiaopeng Liu, Alex J. Battiste, Miriam H. Bregman – *Douglas Mitchell Research Group, Vanderbilt University*

Multinuclear iron-dependent oxidative (MNIO) enzymes are a class of peptide-modifying enzymes involved in the biosynthesis of ribosomally synthesized and post-translationally modified peptide (RiPP) natural products. A recently published report details how one member of this enzyme family transforms Cys residues in precursor peptides bearing -EGKCG- motifs into oxazolone/thioamide moieties. By analyzing closely related homologs of this enzyme which also act on similar substrate motifs, we provide spectroscopic evidence to suggest that the formed post-translation modification is in fact a (5-thio)-oxazole rather than the oxazolone/thioamide structure. Mass spectrometry demonstrated that the (5-thio)-oxazoles effectively bind metals, thereby suggesting the functional utilization of these natural products as defense against metal-induced stress. Currently ongoing work is exploring the role of these gene clusters in evasion from metal-mediated immune responses.

Computational structure prediction of lanthipeptides with NMR data reveals underappreciated peptide flexibility and applications to neuropeptide Y receptor lanthipeptide design
Claiborne W. Tydings, Allison S. Walker – *Jens Meiler Research Group, Vanderbilt University*

Our inability to computationally model and design lanthipeptides in molecular modeling and design software such as Rosetta limits our ability to rationally design lanthipeptides for drug discovery campaigns. Lanthipeptides are a class of thioether containing ribosomally synthesized and post-translationally modified peptide which often have antibiotic activity and interest in using lanthipeptides for bioengineering applications is growing. We propose that implementing support for the lanthionine rings and dehydrated amino acids found in lanthipeptides will enable accurate lanthipeptide modeling with Rosetta. We find that when compared to the ensembles of lanthipeptides with NMR determined structures in the PDB, lanthipeptide ensembles generated with Rosetta have similar experimental agreement, lower Rosetta energy scores, and greater flexibility. Our use of ensemble averaged NOE distances instead of requiring individual structures to satisfy all NOE restraints was key for revealing the flexibility of these peptides. Our Rosetta lanthipeptide ensembles primarily show increased flexibility in non-cyclized peptide regions as well as increased lanthionine ring flexibility when conformationally stabilizing elements are not present. Support for lanthipeptides in Rosetta enables the design and modeling of lanthipeptides in Rosetta for therapeutic development.
We applied this technology to design peptides with anti-obesity potential by targeting the neuropeptide Y receptor family. In humans, this family consists of receptors Y1, Y2, Y4, and Y5. These receptors play critical roles in regulating feeding behavior and energy homeostasis. For example, Activation of Y4 or Y2 promotes satiety, while activation of Y1 or Y5 promotes appetite. Y2/Y4 selective dual agonists could be promising candidates for obesity and diabetes therapeutics. Sidechain conjugated cyclic peptides, including lanthipeptides are attractive class of engineerable Y receptor agonists as they are protease resistant and maintain a free carboxy terminus, critical for Y receptor binding. Accordingly, we have designed the first Y2/Y4 selective dual agonists with nanomolar range activity at both receptors.

Small Molecule Modulation of PMP22 Expression and Trafficking
Mason Wilkinson, Katherine Stefanski, Katherine Clowes, Pramod Gowda, Thomas Hasaka, Emily Days, Josh Bauer, Bruce Carter – *Charles Sanders Research Group, Vanderbilt University*

Mutations in the peripheral myelin protein 22 (PMP22) gene can result in Charcot-Marie-Tooth disease types 1A and 1E (CMT1A and CMT1E), hereditary neuropathy with liability to pressure palsies (HNPP), or Dejerine–Sottas syndrome (DSS). CMT1A and HNPP occur when Schwann cells produce too much or too little PMP22, respectively, which leads to abnormalities in the myelin sheath. We hypothesize that chemical compounds that selectively increase or decrease PMP22 levels in Schwann cells could be applicable to developing treatments for CMT1A, HNPP, and possibly some cases of CMT1E/DSS. Because no FDA-approved treatments currently exist for any of these diseases, we sought to screen for small molecule compounds that can alter PMP22 levels in Schwann cells. To this end, we developed a rat Schwann cell (RSCs) line that can express human PMP22 on demand when we add the antibiotic doxycycline to their growth medium. We first grew these cells at large scale and treated individual populations each with one of 22326 small molecule compounds. Next, we evaluated each compounds effects on Myc-hPMP22 levels at the RSC plasma membrane using high-throughput fluorescence microscopy. Following extensive curation, we have identified three hit compounds that dramatically and specifically alter Myc-PMP22 protein levels without causing cytotoxicity and without perturbing transcription from the exogenous locus. At low micromolar treatment conditions, two compounds largely eliminate Myc-hPMP22 protein levels, while the third nearly doubles the levels measured in untreated cells. We are now pursuing structure-activity relationship experiments to identify more potent chemical analogues of these hits, as well as evaluating their to alter endogenous PMP22 expression and rectify myelination in rat dorsal root ganglion explants.

From Shape Consensus to Stability: Computational Pipeline for Ligand Discovery in the KCNQ1 VSD
Sophia Hamza – *Jens Meiler Research Group, Vanderbilt University*

Long QT Syndrome (LQTS), particularly type 1 (LQT1), arises from mutations in the KCNQ1 gene that impair cardiac repolarization and elevate the risk of fatal arrhythmias during physical exertion. Although previous work has explored the impact of KCNQ1 variants on channel function and expression, the potential for small molecules to bind within structural pockets of the voltage-sensing domain (VSD), particularly in the "down" state (PDB ID: 8SIN), remains unclear. This study investigates whether the VSD pocket can accommodate small molecules with favorable binding properties. Ligands previously docked into the VSD site were used to sample the pocket space, and shape-based screening via ShapeFlex was conducted against a custom ligand library provided by Vanderbilt University and an Enamine database to identify a set of top shape-matched candidates. Prospective ligands were redocked into the VSD pocket using RosettaLigand and rescored based on interface energy. To assess unbiased binding potential, blind docking was performed with DynamicBind to evaluate whether ligands would independently localize within the VSD region. Additional analyses included evaluation of binding interactions, molecular characteristics, and convergence of docking poses, with quantum mechanical rescoring (RosettaQM) applied for enhanced energy evaluation. Several ligands from each screen showed favorable overall binding characteristics and physicochemical properties, as predicted by computational modeling based on their chemical composition. These compounds exhibited features consistent with pocket engagement while spanning a range of distinct chemical scaffolds. Ongoing molecular dynamics simulations are being used to assess the stability of these complexes, and prioritized compounds will be pursued in NMR-based binding assays to determine in vitro engagement.

Targeting Metabotropic Glutamate Receptor 7 to Correct Cognitive Phenotypes in Neurofibromatosis Type 1
Harrison Parent, E. Harriott, S. Chenareddy, A. Taylor, N.M. Fisher, B.J. Stansley, R.R. Lavieri, J.K. Shirey-Rice, J.M. Pulley, R.G. Gogliotti, L. Cutting – *Colleen Niswender Research Group, Vanderbilt University*

Metabotropic glutamate receptor 7 (mGlu7) is a group III metabotropic glutamate (mGlu) receptor regulating glutamate and GABA release which is essential for various learning and memory processes1,2. mGlu7 dysfunction is associated with neurodevelopmental and cognitive impairments including ADHD, seizures, and developmental delay3. A Phenome-wide association study (PheWAS) conducted through the Vanderbilt Institute for Clinical and Translational Research (VICTR) detected a novel association between an expression-attenuating single nucleotide polymorphism (SNP) in the GRM7 gene (rs9870680) and Neurofibromatosis Type 1 (NF1). NF1 is a neurodevelopmental disorder characterized by skin pigmentation abnormalities, benign tumor formation, and cancer predisposition. However, nearly 80% of patients suffer from

cognitive impairments in the domains of working memory, visuospatial skills, attention, and reading[4,5]. Preclinical[9,10] and clinical findings[11,12] suggest that NF1-related cognitive impairments are driven by excessive GABAergic signaling, and the cognitive profiles of NF1 patients and patients with impaired mGlu7 function have considerable overlap. However, there are no currently approved therapies to treat NF1-related cognitive symptoms, and no currently approved mGlu7-targeting drugs. Due to the associations of mGlu7 with learning deficits and GABAergic regulation, we hypothesize that mGlu7 acts as a modifier and potential therapeutic target for cognitive phenotypes in the NF1 population. To test this hypothesis, we are utilizing a combination of in vitro, ex vivo, and in vivo studies, along with human subjects' studies. This multifaceted approach will enhance our understanding of the correlation between mGlu7 and NF1, establish a molecular mechanism of interaction between these cellular signaling pathways, and evaluate the potential of mGlu7 as a therapeutic target for NF1-associated cognitive symptoms.

1. Optical Biosensor Development for Real-Time Imaging of GPR88 Activation in the Striatum

Salisha Baranwal, Edwin Matthiessen, Prashant C. Donthamsetti

G protein coupled receptors (GPCRs) represent the largest superfamily of membrane proteins (>800 members) and are targeted by ~35% of clinically approved drugs. Among these receptors, GPR88 has gained considerable attention due to its near exclusive expression in the striatum, a brain structure that plays key roles in diverse central nervous system disorders (schizophrenia, addiction, Parkinson's Disease). Recent pharmacological and genetic studies indicate that GPR88 can regulate striatum-associated behaviors (motor control, reward processing, and learning). However, GPR88 is a member of a subset of GPCRs called orphan receptors, for which no endogenous ligand has been identified. Thus, little is known about the relationship between the activation dynamics of GPR88 and its corresponding role in behavior, limiting our understanding of the biological role and clinical potential of this receptor. To address this problem, I am developing a novel optical biosensor to measure changes in GPR88 activation in vivo. Prior structural studies indicate that upon activation, GPR88 undergoes a conformational change wherein its transmembrane segments 5 and 6 (TM5 and TM6) move apart, allowing signaling proteins to bind the receptor. Using a structure-guided analysis of GPR88, I replaced the third intracellular loop that connects TM5 and TM6 with the environmentally sensitive fluorophore circularly permutated green fluorescent protein (cpGFP). In proof-of-concept studies, the synthetic positive allosteric modulator 2-PCCA induces a change in the fluorescence of a GPR88-cpGFP prototype, confirming that this biosensor functions as intended. To enhance the dynamic range, I generated variants with randomized linkers flanking the cpGFP insertion site in GPR88 and screened their function using a novel high-throughput plate-reader based assay. Further validation using confocal microscopy indicated that GPR88-cpGFP can detect dynamic changes in GPR88 activity. Taken together, these studies could improve our understanding of GPR88 and provide a template for interrogating other elusive orphan receptors in living animals.

2. Enabling Advanced Metabolomic and Lipidomic Research Through Mass Spectrometry

The Center for Innovative Technology (CIT)

The Center for Innovative Technology (CIT) provides researchers with access to cutting-edge mass spectrometry platforms for metabolomic and lipidomic analyses. In collaboration with the McLean Laboratory, the CIT has been recognized as a Waters Center of Innovation (Waters Corporation) and an Agilent Thought Leader Laboratory (Agilent Technologies). Our multidisciplinary team brings over 50 years of collective expertise in mass spectrometry, data processing, and informatics. CIT supports a wide range of research goals by offering both routine and advanced molecular profiling services. Emphasizing global untargeted analyses, CIT enables researchers to assess steady-state or dynamic changes in complex metabolite populations across various biological matrices, including fluids, cells, and tissues. Metabolite identification is achieved using accurate mass-to-charge measurements, chromatographic retention times, isotopic distribution patterns, tandem MS/MS fragmentation, and spectral alignment with curated in-house libraries. These high-resolution datasets uncover distinct biochemical signatures that reflect underlying cellular processes within specific biological systems. As a result, CIT's analytical capabilities empower researchers to generate new hypotheses and deepen understanding of metabolic pathways modulated by genetic perturbations, environmental exposures, or disease states.

3. Comparing MALDI Imaging Platforms: A Systematic Evaluation of Lipid Imaging Performance

Megan S. Ward, Madeline E. Colley, Martin Dufresne, Ali Zahraei, Lukasz G. Migas, Melissa Farrow, Raf Van de Plas, Jeffrey M. Spraggins

Matrix-assisted laser/desorption ionization imaging mass spectrometry (MALDI IMS) is an established tool for imaging lipids in complex biological samples. MALDI IMS enables highly multiplexed molecular imaging in a single experiment. For example, hundreds of phospholipids can be mapped in situ using standard sample preparation methods. Since MALDI IMS lacks pre-ionization separations, it produces complex mass spectra. To improve specificity and molecular coverage, ultra-high mass resolving power instruments (e.g., Fourier-transform mass spectrometry) and ion mobility (e.g., trapped ion mobility spectrometry (TIMS)) have previously been employed. Here, we present a systematic study of lipid imaging performance afforded by different MALDI IMS platforms, quantifying the effects mass resolving power and gas-phase separations have on key analytical figures of merit.

HeLa cells were grown on indium tin oxide (ITO) coated slides at 37°C and 5% CO2 in Dulbecco's modified eagle medium (DMEM) with 10% fetal bovine serum (FBS). Cells were allowed to proliferate overnight. Afterward, HeLa cells were washed with Dulbecco's phosphate-buffered saline (DPBS) and chilled 150 mM ammonium formate. Autofluorescence and brightfield images were acquired via a Zeiss AxioScan.Z1 slide scanner. VANDY 37, a custom synthesized matrix, was sublimated onto the cells using an HTX SubliMATE. Data was acquired with a 15T solariX FT-ICR equipped with a customized Smartbeam II 2 kHz Nd:YAG (355 nm) laser with a Gaussian profile and timsTOF fleX. Data was analyzed using Bruker Daltonics' DataAnalysis, SCiLS Lab, and ftmsProcessing and in-house developed software.

Dispersed HeLa cells were imaged on a timsTOF fleX equipped with a microGRID stage at a spectral resolving power of 40,000. HeLa cells were imaged with a 5 µm pitch, which was verified via post-MALDI IMS autofluorescence and brightfield microscopy. Additionally, HeLa cells were imaged with a 50 µm pitch using a 15T FT-ICR at 40,000 resolving power at m/z 622. This provided a baseline comparison of molecular coverage for the two mass spectrometers. Increasing the resolving power to 350,000 at m/z 622 using the 15T FT-ICR allowed for the separation of isotopic envelopes and isobars, resulting in increased molecular feature detection. Additionally, TIMS was performed using 100 ms and 800 ms ramp times on the timsTOF flex platform, improving our ability to differentiate nominally isobaric molecular features, resulting in higher-quality ion images. These experiments were performed in both positive and negative ion modes. To validate lipid annotations, microflow reverse-phased LC-MS/MS was performed using a Waters M-Class UHPLC. Mobile phase A was composed of 60:40 ACN:H2O with 5 mM ammonium acetate and 0.1% formic acid. Mobile phase B was composed of 80:15:5 IPA:ACN:H2O with 0.1% FA and 5mm ammonium formate. A 4D lipidomic method with MS/MS stepping enabled was employed, and data was searched using MS-DIAL. We detected phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylserine lipid classes in both MALDI IMS and LC-MS/MS experiments. We quantify quantify key analytical metrics, including lipid-class molecular coverage, structural specificity, image quality, and imaging quality to provide a comprehensive platform comparison.

Systematic quantification of lipid imaging performance across multiple MALDI platforms, highlighting differences in resolution, molecular coverage, and ion mobility separations

4.

Abigail Rose

Life on this planet began and thrived in the absence of oxygen for billions of years until photosynthesis released molecular oxygen into the atmosphere, which led to extinction of > 95% of anaerobic organisms. While some organisms evolved to thrive in the O2-rich environment, others dwell in habitats that are devoid of oxygen, including the human gastrointestinal tract. Although anaerobes as a life form have been discovered for over 150 years, little is known about why this molecular oxygen constitutes such a lethal challenge to these organisms. In addition

to shedding light on the fundamental biological question, a better understanding of the mechanisms underlying oxygen lethality in anaerobes will have significant impact on improving human health, as oxygen becomes available during gut inflammation, depleting beneficial anaerobic commensal bacteria Here we utilize chemoproteomics and genome-scale metabolic modeling to identify enzymes that are damaged in the presence of O2 within an obligate anaerobic gut commensal, Bacteroidetes thetaiotaomicron. The identified oxygen-damaged B. thetaiotaomicron enzymes were then replaced with oxygen-tolerant equivalents from facultative anaerobic bacteria. The resultant engineered B. thetaiotaomicron strain outcompetes the wild-type during in vitro O2 exposure and exhibits improved resilience during inflammation elicited by proinflammatory pathogen Salmonella Typhimurium. We demonstrated that the increased fitness of our engineered strain during O2 exposure is the result of restored central metabolic activity and increased reducing power to neutralize the ROS produced upon O2 exposure. The rational engineering of an obligate anaerobic bacteria to withstand oxygen simultaneously allows us to unravel the principles of obligate anaerobiosis and establish the framework for engineering an O2-resistant probiotic to mitigate dysbiosis during intestinal inflammation.

5. From Shape Consensus to Stability: Computational Pipeline for Ligand Discovery in the KCNQ1 VSD

Sophia Hamza

Long QT Syndrome (LQTS), particularly type 1 (LQT1), arises from mutations in the KCNQ1 gene that impair cardiac repolarization and elevate the risk of fatal arrhythmias during physical exertion. Although previous work has explored the impact of KCNQ1 variants on channel function and expression, the potential for small molecules to bind within structural pockets of the voltage-sensing domain (VSD), particularly in the "down" state (PDB ID: 8SIN), remains unclear. This study investigates whether the VSD pocket can accommodate small molecules with favorable binding properties. Ligands previously docked into the VSD site were used to sample the pocket space, and shape-based screening via ShapeFlex was conducted against a custom ligand library provided by Vanderbilt University and an Enamine database to identify a set of top shape-matched candidates. Prospective ligands were redocked into the VSD pocket using RosettaLigand and rescored based on interface energy. To assess unbiased binding potential, blind docking was performed with DynamicBind to evaluate whether ligands would independently localize within the VSD region. Additional analyses included evaluation of binding interactions, molecular characteristics, and convergence of docking poses, with quantum mechanical rescoring (RosettaQM) applied for enhanced energy evaluation. Several ligands from each screen showed favorable overall binding characteristics and physicochemical properties, as predicted by computational modeling based on their chemical composition. These compounds exhibited features consistent with pocket engagement while spanning a range of distinct chemical scaffolds. Ongoing molecular dynamics simulations are being used to assess the stability of these complexes, and prioritized compounds will be pursued in NMR-based binding assays to determine in vitro engagement.

6. Mapping the Proteostasis Network of Adenosine Deaminase 2 (ADA2) Variants associated with Deficiency of Adenosine Deaminase 2 (DADA2) using AP- (DIA) MS

Lea A. Barny, Minsoo Kim, Lars Plate

Deficiency of adenosine deaminase 2 (DADA2) occurs due to mutations in the ADA2 protein, resulting in symptoms ranging from vascular inflammation to bone marrow failure. Precise insights into the cellular biogenesis of ADA2 are lacking which hampers development of effective treatments. Here, we used affinity purification-mass spectrometry (AP-MS), with DIA-MS acquisition, to map the protein homeostasis (proteostasis) network in ADA2 variants. Secretion deficient variants (R169Q and Y453C) showed the greatest increase in interactions with protein folding machinery, whereas mis-localizing variant (R34W) showed highest interactions with cytosolic degradation factors. A loss of activity variant (T360A) demonstrated decreased interactions compared to wild-type ADA2 whereas a gain of activity variant (K400D) exhibited interactions resembling those of wild-type ADA2. We additionally conducted time-resolved interactome profiling (TRIP) during the biogenesis of ADA2 to elucidate the timing of steady-state interactions with these ADA2 variants. To investigate the impact of these interactors, we performed siRNA-mediated knockdown (KD) of the identified interactors, measuring rescue of secretion through a nano-luciferase luminescence assay and activity using an indophenol colorimetric assay. Knockdown of TEX264 and DERL1, for example, resulted in both an increase in secretion and activity of the Y453C variant, indicating modulation of the proteostasis network can result in correctability of ADA2. Our findings enhance the understanding of ADA2 misfolding and quality control, revealing alternative cellular targets for treating DADA2. Additionally, our assay systems serve as valuable platforms for drug screening.

7. Cleave and Let Die – Targeting H. pylori Gyrase with Novel Antibacterial Classes

Aleksya Drobshoff, Jillian Armenia, Neil Osheroff

Helicobacter pylori, a Gram-negative spiral bacterium, is a causative agent of chronic gastritis, peptic ulcers, and gastric cancer that affects nearly half of the world population. Fluoroquinolones are a common class of antibacterials used to treat H. pylori. The primary molecular targets of fluoroquinolones are the bacterial type II topoisomerases, gyrase and topoisomerase IV. Unfortunately, target-mediated fluoroquinolone resistance in H. pylori is on the rise and has diminished the effectiveness of this class as front-line drugs for this infection. Two new classes of type II topoisomerase-targeting antibacterials, triazaacenapthylenes and spiropyrimidinetriones, have recently emerged and both show promise for the treatment of fluoroquinolone-resistant infections. Gepotidacin (a triazaacenapthylene) was recently FDA approved for the treatment of uncomplicated urinary tract infections and is a late-stage clinical candidate for gonorrheal infections. Zoliflodacin (a spiropyrimidinetrione) was just accepted for priority review by the US FDA for the treatment of gonorrheal infections. To evaluate the efficacy and mechanism of enzyme inhibition of current and novel type II topoisomerase-targeting compounds against H. pylori, in vitro assays were performed to measure their impact on gyrase-mediated DNA cleavage and supercoiling. Preliminary results indicate that fluoroquinolones, moxifloxacin, and ciprofloxacin induce ~30% double-stranded breaks with high potency (EC_{50} < 1 μM). Zoliflodacin results in ~40% single-stranded breaks and ~30% double-stranded breaks but with lower potency (EC_{50} ~8 μM), while gepotidacin generates ~30% single-stranded breaks and exhibits the highest potency (EC_{50} < 0.1 μM). All four compounds also inhibited the catalytic activity of gyrase. However, their potency was reduced compared to DNA cleavage enhancement. IC_{50} values for supercoiling inhibition were all >10 μM. These findings highlight the potential of triazaacenapthylenes and spiropyrimidinetriones as promising alternatives for treating infections caused by H. pylori. Ongoing studies are examining the effects of these compounds on fluoroquinolone-resistant H. pylori gyrase. Supported by NIH Grants R01GM126363, R01AI17054, T32AI11254 and, T32AI112541.

8. Site-specific protein backbone thioamidation in bacteria

Andrew Rice

Despite consisting of a single atom substitution, transformation of a backbone amide to a thioamide can result in significant effects. Nature has utilized this strategy for the post-translational modification of various peptides and proteins. Here, we demonstrate that the E. coli ribosome, specifically subunit uL16, is thioamidated by a misannotated YcaO enzyme. We not only establish the prevalence of this modification throughout Nature through bioinformatic analysis coupled to this uniquely acidic enzyme, but also elucidate the uncharacteristic substrate-enzyme interaction between EcuL16 and EcYcaO. Rather than recognizing solely the local peptide sequence, as all other YcaO enzymes do, EcYcaO recognizes the tertiary structure of EcuL16b for site-selective thioamidation. A robust proteome-wide Alphafold3-based analysis is leveraged to assess potential interacting proteins across the whole organism. This work establishes a body of work upon which further inquiries can be assessed, including the mechanism of sulfur transfer, the functional underpinnings of such a unique post-translational modification,

and whether other proteins in other organisms are harboring such a seemingly innocuous modification.

9. Activation of metabotropic glutamate receptor 3 (mGlu3) corrects schizophrenia-like glutamatergic abnormalities in corticolimbic circuitry and cognitive deficits

Shalini Dogra, Arisa Timoll, Niki Harris, P. Jeffrey Conn, Colleen M. Niswender

Accumulating clinical evidence suggests that an imbalance of excitatory-inhibitory neurotransmission in the prefrontal cortex (PFC) may lead to cognitive deficits. In this context, metabotropic glutamate receptor 3 (mGlu3) has emerged as a compelling target for modulating excitatory signaling in the PFC. Polymorphisms in GRM3 are linked with reduced performance in cognitive tasks in schizophrenia patients and preclinical studies suggest that increasing mGlu3 activity may enhance cognition. However, the mechanism of mGlu3 activation is not fully understood and is further characterized within our study. Cortical neurons projecting to the nucleus accumbens (NAc; mPFCNAc) were traced by injecting viral particles encoding green fluorescent protein (GFP) in the NAc and were voltage clamped to record spontaneous excitatory or inhibitory postsynaptic currents in the presence of mGlu2/3 agonist (LY379268), mGlu2 negative allosteric modulator (VU6001966), and mGlu3 negative allosteric modulator (VU6010572). Cognitive impairments were assessed in vivo using a Y-maze test of spontaneous alternations. We found that MK801 enhanced the excitatory-inhibitory (E/I) ratio in mPFCNAc neurons. Bath application of LY379268 reduced MK801-induced increases in E/I ratio and effects were blocked by the mGlu3 NAM, suggesting that mGlu3 mediates the efficacy of LY379268 in restoring E/I balance in MK801-treated mice. Further, activation of mGlu3 corrected MK801-induced increases in glutamatergic neurotransmission in the NAc, suggesting that mGlu3 can correct MK801-induced glutamatergic abnormalities in the NAc. We next evaluated the ability of mGlu3 activation to reverse MK801-induced deficits in Y-maze test of cognition. Interestingly, treatment with LY379268 corrected MK801-induced deficits in spontaneous alternations. Further, the effects of LY379268 were blocked by pretreatment with an mGlu3 NAM, suggesting that activation of mGlu3 can rescue cognitive deficits in mice. Together, these studies provide important information about the circuit-specific mechanisms by which activation of mGlu3 may affect cognition in schizophrenia and may provide future directions to develop highly selective mGlu3 agonists as novel therapeutics for schizophrenia.

10. Investigating A1-Ring Physicochemical Influence on Everninomicin Activity via Chemoenzymatic Synthesis

Jennifer Wurm, Audrey Yñiguez-Gutierrez, Brian Bachmann

Natural product secondary metabolites have played a fundamental role in the development of drug candidates to combat the increasing threat of antimicrobial resistance due to their ability to target essential microbial pathways. Everninomicin is a natural product antibiotic that inhibits bacterial protein synthesis through its unique ribosomal binding site and exhibits potent activity against high-risk, multi-drug-resistant bacteria. It was developed under the drug name Ziracin and advanced to Phase III clinical trials, but further development was abandoned due to challenges associated with formulating a version that prevented toxic aggregation. Its complex octasaccharide scaffold, requiring over 130 steps by chemical synthesis, has limited the ability to tailor structural modifications for optimization. To overcome this, our lab developed a chemoenzymatic method to produce everninomicin analogs by utilizing the native biosynthetic machinery of its producing strain Micromonospora carbonacea.

Through large-scale fermentation of our lab's engineered M. carbonacea strain, we isolated Everninomicin Q (Evn Q), a heptasaccharide everninomicin metabolite lacking its terminal fully substituted aromatic ring (A1). With an obtainable starting scaffold in hand, we chemically synthesized a small library of aryl A1 ring derivatives and demonstrated the ability of EvdD1, a KASIII-like enzyme in the everninomicin gene cluster, to catalyze their attachment onto Evn Q. This approach yielded five novel everninomicin analogs with di-substituted A1 rings, all of which inhibited in vitro protein synthesis (IC50 ~1–3 μM). In whole-cell assays, however, the analogs and Evn Q failed to inhibit bacterial growth, in contrast to the Ziracin positive control which bears the native A1 ring. Given these findings, we hypothesize that the A1 ring's significance lies in the fine tuning of the drug's physicochemical properties such as solubility or membrane permeability.

To explore this, we sought to expand our library of A1-ring derivatives to include a variety of physicochemical properties. Anticipating potential limitations in EvdD1's substrate scope, we investigated its ability to install aryl groups bearing clickable handles. This effort successfully yielded an azido-aryl–Evn analog, to which we subsequently attached an alkyl carboxylic acid moiety via copper-catalyzed click chemistry (CuAAC) in a one-pot reaction. Ongoing work focuses on using this strategy to continue installing functional groups onto Evn Q that probe bioactivity-relevant properties. These efforts support our goal of generating broader analog diversity to ultimately guide future optimization of everninomicin's drug-like properties and therapeutic potential.

11. Targeted Reactivation of Frataxin via Synthetic Genome Regulators: Mechanistic Insights into BET Bromodomain Engagement

Ashraf Mohammed

Friedreich's ataxia (FA) is a progressive neurodegenerative disease caused by transcriptional silencing of the FXN gene due to GAA trinucleotide repeat expansions. We developed synthetic genome readers/regulators (SynGRs), such as SynTEF1, which are bifunctional molecules composed of a sequence-specific DNA-binding polyamide tethered to a pan-BET ligand (e.g., JQ1). These constructs recruit BET family transcriptional coactivators to the silenced FXN locus to restore gene expression. To explore the mechanistic basis of BET engagement, we systematically evaluated SynGRs bearing bromodomain-selective ligands. Surprisingly, only SynGRs with pan-BET or BD2-selective ligands activated FXN transcription, while BD1-selective SynGRs were inactive in cells despite their high in vitro affinity for BD1. However, co-treatment with free BD1-selective ligands like GSK778 enhanced SynGR activity, revealing competitive BET protein dynamics between natural transcription factors and synthetic recruiters. Further structure-guided redesign of the chemical linker in BD1-selective SynGRs restored activity, pinpointing a critical spatial configuration necessary for productive chromatin engagement via BD1. These findings demonstrate that SynGRs mimic natural transcription factor behavior rather than functioning as chromatin mimics. Our work provides new insights into domain-selective BET recruitment and establishes a platform for the rational design of gene-targeted therapeutics.

12. Structure-Based Discovery and Definition of RiPP Recognition Elements

Miriam H. Bregman, Dillon P. Cogan, Kyle E. Shelton, Andrew J. Rice, Shravan R. Dommaraju, Satish K. Nair, Douglas A. Mitchell

Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a diverse class of natural products with wide-ranging activities and synthetic potential. Central to many RiPP biosynthetic pathways is the RiPP Recognition Element (RRE), a structurally conserved peptide-binding domain that enables class-independent genome mining. While RRE-Finder has leveraged this feature to identify novel RiPPs, its accuracy has been limited by false positives, particularly due to structural similarities with unrelated protein families like transcription factors. To address these limitations, we integrated AlphaFold 3-based structure prediction with rapid tertiary structure alignment using Foldseek. This approach revealed many sequence-divergent RREs missed by the original RRE-Finder models. We used these to build 11 new Foldseek-derived Hidden Markov Models (HMMs) and refined existing models through improved seed alignments and domain excision. We also implemented model-specific bitscore thresholds and removed over 196 PFam annotations found to contribute to false positives based on structural context. These updates expanded Precision mode from 29 to 59 HMMs and recovered many novel RREs not identified in RRE-Finder v1, including proteins fused to domains not previously associated with RiPP biosynthesis. In total, the updated workflow identifies 91,283 putative RREs. To further characterize these RREs and assess their functional relevance, we used AlphaFold 3 to predict over 10,000 RRE–peptide complexes. This enabled the mapping of 13 distinct recognition motifs across known RiPP classes, including one novel motif that was validated in vitro alongside two additional predicted interactions. Together, these improvements enhance both the specificity

and scope of RRE-Finder, enabling more confident identification of novel RiPP precursors and improving access to previously undetectable biosynthetic pathways.

13. Characterizing coronavirus nonstructural protein 2 (nsp2) interaction with host translation machinery
Brynn Roman, Lars Plate

Nonstructural proteins (nsps) play a crucial role setting up RNA viral replication in the infected host cell and allowing viruses to exert control over host cellular processes. Exerting control over host translation machinery is necessary during the viral life cycle to slow endogenous host protein synthesis while promoting viral protein translation. Previous studies in the Plate group identified nonstructural protein 2 (nsp2) in SARS-CoV-1 (SARS1) as a viral protein that interacts with translation repressors GIGYF2 and 4EHP. 4EHP is a 5' mRNA cap-binding protein that inhibits translation initiation while GIGYF2 stabilizes the 4EHP/mRNA cap interaction. 4EHP is homologous to 5' mRNA cap-binding protein EIF4E1, however it is unable to initiate translation. The identified host-virus protein interactions suggest nsp2 interferes with protein translation. Surprisingly, SARS-CoV-2 (SARS2) nsp2 does not strongly interact with 4EHP and GIGYF2, despite the SARS1 and SARS2 homologs having a sequence similarity of 79.1%. I hypothesize SARS1 nsp2 interacts with 4EHP and GIGYF2 to inhibit host translation of specific transcripts. The underlying mechanism by which SARS1 nsp2 regulates protein translation and influences the function of 4EHP and GIGYF2 is not understood. Elucidating the mechanism and function of this strain-specific interaction may provide insight into the divergent pathogenicity observed between SARS1 and SARS2. Using co-immunopurification to enrich SARS1 or SARS2 nsp2 coupled to LC-MS/MS has allowed for comparison of the homologs' interactomes, comparison of the homologs' effects on the host cell proteome, and identification of the region of nsp2 mediating 4EHP/GIGYF2 binding capability.

14. Cation diffusion facilitator proteins modulate C. difficile oxidative stress resistance
Summer D. Bushman, Eric P. Skaar

Clostridioides difficile is an enteric pathogen and a leading cause of hospital-acquired diarrhea. As an obligate anaerobe, vegetative C. difficile cells cannot survive exposure to oxygen, and instead form oxygen-resistant spores. Manganese, as both an element and a protein cofactor, is essential for bacteria to resist oxidative damage generated by the vertebrate immune system. Manganese and other nutrient metals, such as iron and zinc, are essential for life, but they are toxic at high concentrations. During bacterial infections, vertebrate hosts modulate metal levels to mitigate infections in a process termed nutritional immunity. Bacteria have evolved mechanisms to maintain metal homeostasis, which are often under the control of metalloregulatory proteins, such as the ferric uptake regulator (Fur). The involvement of zinc and iron during C. difficile infection has been explored, however the role of manganese in the context of C. difficile infection has been understudied. Using comparative genomics, genes encoding proteins hypothesized to transport metals were identified in the C. difficile genome, including the identification of four different genes that are predicted to encode cation diffusion facilitator (CDF) family proteins. Two of these CDF genes, CD196_RS09020 and CD196_RS17790 (17790), have been previously reported to be induced by Fur, suggesting that they influence metal homeostasis. Here, we show that 17790 protects Staphylococcus aureus against manganese toxicity, suggesting 17790 is a manganese efflux pump. Furthermore, 17790 C. difficile has higher intracellular manganese levels compared to wildtype (WT), as measured by inductively coupled plasma mass spectrometry. Given that manganese impacts oxidative stress resistance, WT and 17790 strains of C. difficile were tested for their ability to survive hydrogen peroxide exposure. The 17790 mutant is more resistant to hydrogen peroxide than WT C. difficile. Collectively, these data suggest 17790 modulates manganese homeostasis and oxidative stress resistance within C. difficile, which may influence pathogenesis.

15. Protein Conformational Landscape Prediction with Quantum Computers
Katy Butler, Mosavverul Hassan, Benjamin P. Brown

Protein functions emerge as a consequence of conformational motion. Deep learning models such as AlphaFold2 have revolutionized protein structure prediction by leveraging evolutionary information from multiple sequence alignments (MSA); however, these models have been designed to rapidly reconstruct valid all-atom structures representing only the highest probability conformation. In the context of MSA-based methods such as AF2, this means that generating a conformational ensemble requires input manipulation, such as perturbing the MSA, to sample distinct structural states. It has been proposed that AF2 uses MSA-driven components to locate the approximate global minimum on a learned energy surface, with later stages acting as an energy function for local refinement. We posit a variant of this view: that the probabilistic residue-pair distance map (distogram) produced by AF2 itself is representative of an effective free energy landscape for the protein, and that perturbing the MSA constitutes a perturbation of the underlying Hamiltonian.
Our perspective redefines the sampling task: we aim to comprehensively sample the low-energy conformational ensemble consistent with a single distogram. To achieve this, we develop a hybrid quantum-classical framework that formulates the conformational sampling as a Quadratic Unconstrained Binary Optimization (QUBO) problem. The QUBO Hamiltonian encodes the AF2-derived energy landscape: linear terms represent the probabilities of discretized inter-residue distances from the distogram, while quadratic penalty terms enforce physical constraints. These constraints include ensuring a unique distance is assigned to each residue pair and maintaining proper geometry between adjacent residues. To enforce global geometric consistency, we systematically penalize violations of the triangle inequality. These three-body interactions are reduced to the required quadratic form by introducing auxiliary variables. Low-energy solutions from the final QUBO, which are sets of self-consistent Cβ distances, are obtained from quantum annealing. The final collection of states are reconstructed into full, all-atom models by first solving a classical distance-geometry problem (DGP), followed by quaternary kinematic backbone reconstruction (QBKR) to generate a backbone trace with Cβ atoms, and then by sidechain packing with Rosetta. Our approach establishes a framework to harness quantum annealers for complex sampling problems in structural biology, translating learned structural embeddings into dynamic conformational ensembles.

16. Phase separation regulates zinc homeostasis in a gut commensal
Deepanshu Singla, Wenhan Zhu

The resilience of commensal gut bacteria is critical for preserving host health during inflammatory insults, yet the molecular mechanisms that enable their survival under such stress remain poorly understood. A hallmark of intestinal inflammation is host-mediated zinc sequestration, which limits microbial access to this essential micronutrient. Here, we uncover a novel phase-separation-based mechanism that enables the human gut commensal Bacteroides thetaiotaomicron (B. theta) to regulate zinc homeostasis and persist in the inflamed gut. We identify a zinc-responsive transcription regulator, ZusA, which undergoes liquid–liquid phase separation under zinc-replete conditions to terminate expression of the high-affinity zinc transporter ZusBC. Under zinc-limiting conditions, this regulatory mechanism is suppressed, allowing zinc acquisition and promoting bacterial survival. These findings reveal phase separation as a previously unrecognized strategy for commensal adaptation and resilience during inflammation, offering new insights into microbiota stability and potential therapeutic avenues for inflammatory diseases.

17. Discovery of Highly Anionic RiPPs from Aquatic Bacteria
Dominic Luciano, Shravan R. Dommaraju, Hamada Saad, Douglas A. Mitchell

The whole-genome shotgun database contains an abundance of genomic sequences from unannotated environmental samples. This vast sequence space is predicted to harbor insights into unexplored ecological niches from uncultured bacteria, novel metabolic pathways, and molecules with therapeutic potential. To access this untapped resource, RODEO was

redesigned to annotate metagenomic datasets, resulting in MetaRODEO. Using MetaRODEO, 51,000 BioProjects were analyzed, revealing new genomic contexts for RiPPs that contain RiPP Recognition Element (RRE) domains. To validate MetaRODEO's accuracy, a cyanobacterial lanthipeptide was successfully produced via heterologous expression. Further support came from the understudied phylum Acidobacteria, where MetaRODEO identified RiPPs featuring a prenylated peptide from a divergent rSAM enzyme and a lasso peptide.
Mining RRE-associated gene neighborhoods revealed a wealth of putative RiPPs in aquatic environmental samples. Notably, a highly phosphorylated peptide was identified from a hydrothermal vent metagenome, while another peptide—heavily modified with γ-aminobutyric acid (GABA)—was discovered in samples from saline-alkaline lakes. These peptides share a highly anionic C-terminus: the former contains up to 14 phosphorylation events, and the latter up to 17 GABAylations. The effective extension of the carboxylate group by GABA in these GABAtides, along with their restriction to aquatic Gammaproteobacteria from saline-alkaline lakes, suggests an ecological role that is currently under investigation. Interestingly, GABA is installed by a divergent tubulin tyrosine ligase-like (TTLL) enzyme—previously unobserved in prokaryotes—indicating a potential co-option of this eukaryotic enzyme to modify a tubulin-like RiPP precursor peptide that mirrors the C-terminus of eukaryotic tubulin.

18. Prediction and Discovery of Elicitor/Regulator Pairs Using a Heterologous Expression System
Emilee Patterson

Antimicrobial resistance (AMR) is an escalating global health threat, contributing to a growing number of infections and deaths worldwide. Although clinical strategies such as stewardship programs and patient education help address AMR, the discovery of new drugs remains critical for treating resistant infections. Natural products are a class of drugs often derived from bacteria, such as the soil dwelling genus Streptomyces. These bacteria are a rich source of natural products due to their large genomes and numerous biosynthetic gene clusters (BGCs) responsible for producing bioactive compounds. However, many of these clusters are silent under standard laboratory conditions, preventing access to their full biosynthetic potential. In addition, BGC expression is metabolically costly so their activation must be tightly regulated by specialized control systems. One such example are TetR proteins, a family of BGC regulators with a characteristic N-terminal helix-turn-helix DNA-binding domain that inhibits transcription by blocking access to DNA. Their C-terminal domain binds small molecules, or elicitors, which control their DNA-binding activity. When an elicitor binds to a TetR, the protein releases from DNA, allowing transcription to proceed. If the elicitor for a TetR is known, this mechanism can be exploited to induce targeted expression of the BGC it regulates. While thousands of TetRs have been identified, very few of their elicitors have been characterized. This work presents a method for elicitor discovery using the TetR protein JadR2, which has a known elicitor, chloramphenicol. Using sequence similarity networks, I identified homologs of JadR2 that may respond to the same elicitor. I then developed a two-plasmid heterologous expression system using GFP as a readout of JadR2's DNA-binding activity. This system provides a high-throughput method for elicitor screening and offers insight into TetR-DNA dynamics. Using this approach, I identified three JadR2 homologs that are elicited by chloramphenicol. This work offers a simple and scalable method for discovering elicitor/regulator pairs that could be used to activate silent gene clusters and uncover novel natural products.

19. Trypsin Activation of Protease-activated Receptor 4 (PAR4)
Emma Webb, Heidi Hamm

Protease-activated receptor 4 (PAR4) is a GPCR mostly expressed on platelets that leads to platelet activation and aggregation. PAR4 is activated by a tethered ligand mechanism, where the N-terminus of the receptor is cleaved by a protease, which reveals a new N-terminus, dubbed the tethered ligand. The tethered ligand folds back and activates the receptor. PAR4 can also be activated by a peptide mimetic of the tethered ligand sequence, known as the PAR4 agonist peptide (PAR4 AP). When activated, PAR4 signals through Gαq and Gα12/13 G proteins and ß-arrestin. GαQ signaling results in calcium mobilization, Gα12/13 signaling results in cytoskeletal rearrangement, and ß-arrestin recruitment leads to receptor internalization and degradation. Together, PAR4 activation leads to thrombosis, inflammation, and cell migration. Although much is known about PAR4 activation via thrombin cleavage, not much is known about other proteases. Thrombin is thought to be the main activator of PAR4. However, trypsin and Cathepsin G are known proteases that also cleave the N-terminus of PAR4. PAR4 becomes overexpressed in other cell types in inflammatory environments, and it is not known what protease activates the receptor in these conditions. Thus, to better understand various protease activation of the PARs family, we have characterized the activation of PAR4 via trypsin cleavage in human platelets.

20. Evaluating and targeting DNA repair defects in pediatric high grade glioma
Evan Savage

Homologous recombination deficiency (HRD) has been extensively studied in breast cancer and ovarian cancer given its correlation with sensitivity to Poly (ADP-ribose) polymerases (PARP) inhibitors. In these cancers, HRD is most frequently caused by biallelic inactivation of BRCA1/2 and these tumors often exhibit 'genomic scars' (Nik-Zainal, 2016; Telli, 2016; Davies, 2017). Our group profiled a cohort of 255 pediatric high-grade glioma (pHGG) patient samples for DNA damage response (DDR) gene variants and genomic scars. We found no evidence of BRCA1/2 loss in any sample, but we did detect evidence for HRD-associated genomic scars in 30% of our samples. Interestingly, we also detected biallelic loss of ATM, which is reported to be synthetically lethal with ATR inhibition (Marwan, 2016). Given these findings, our group set out to answer the following questions: 1) Does the presence of BRCA deficiency or HRD-related genomic scars predict sensitivity to PARP inhibition in pHGG tumors? 2) Do the ATM mutations we found impair ATM pathway activation and confer radiosensitivity, and do they induce synthetic lethality with ATR inhibition? Overall, this work underscores the importance of looking beyond simple mutation status to identify robust biomarkers of drug response in pHGG tumors.

21. NO Country for B. theta: A Novel sRNA-DNA-Transcription Factor Complex Coordinates Commensal Nitrosative Stress Response
Ryan Fansler, Luisella Spiga, Deepanshu Singla, Daniel Ryan, Alexander Westermann, Adam Deutschbauer, Dan Bak, Wenhan Zhu

The gut microbiota plays a crucial role in human health and disease, influencing the outcomes of both infectious and non-communicable diseases. Invading pathogens or other perturbations elicit intestinal inflammation, which can drive the gut microbiota into an imbalanced state, dysbiosis, that exacerbates inflammatory diseases in susceptible individuals. During episodes of intestinal inflammation, mammalian hosts produce reactive nitrogen species (RNS) to defend against enteric pathogens. Decades of research on model pathogens such as Salmonella have uncovered the guiding principles of bacterial defense against nitrosative stress. However, our understanding of how the commensal microbiota survives nitrosative stress in the inflamed gut remains unstudied. Here, we show that the prominent intestinal commensal Bacteroides thetaiotaomicron employs the sRNA SnoA (sRNA Nitric Oxide regulator A) to orchestrate its nitrosative stress response. This sRNA regulates the expression of the enzymatic effectors nitrite reductase and hybrid cluster protein (Hcp) to detoxify RNS and maintain B. thetaiotaomicron fitness during nitrosative stress in vitro and in vivo. SnoA mediates this regulation by recruiting the transcription factor HcpR to its target promoters, thereby activating their transcription through a DNA-sRNA-transcription factor tripartite complex that has not been previously reported in bacteria. Together, these data illustrate how a prominent gut commensal uses sRNA in a novel mechanism to orchestrate its nitrosative stress response and remain resilient in the inflamed gut.

22. NMR-Based Fragment Screening Targeting the Membrane Protein Peripheral Myelin Protein 22
Geoffrey Li, Thilini Ukwaththage, Charles R. Sanders

Peripheral myelin protein 22 (PMP22) is a tetraspan membrane protein that is highly expressed in compact myelin. Overexpression or missense mutations in PMP22 are associated with Charcot-Marie-Tooth Disease

(CMTD), a hereditary peripheral neuropathy for which no treatment currently exists. This disease is believed to stem from misfolding and aggregation of PMP22. In this study, we employed NMR spectroscopy to screen a fragment library for compounds that bind to PMP22 in vitro and potentially impact its folding and function. We here describe the optimization of methods to apply NMR-based fragment screening to an integral membrane protein target. After screening PMP22 in dodecyl-β-maltopyranoside (DDM) micelles against a subset of the library, we found several compounds that bind to PMP22 in DDM micelles but not to a negative control membrane protein. We subsequently determined the binding affinity of the hits, investigated their structure-activity relationships using NMR and fragment analogs, and used NMR data to restrain modeling of the structures of the hit/PMP22 complexes. Our findings demonstrate the feasibility of NMR-based fragment screening to discover chemical compounds that can bind to a tetraspan membrane protein like PMP22, laying groundwork for future therapeutic strategies in CMTD. This work was supported by a grant from the CMT Research Foundation, by US NIH grant R01 NS095989 and by Deerfield Management Company, L.P. through Ancora Innovation, the Vanderbilt/Deerfield partnership.

23. Comprehensive substrate profiling of a lasso peptide cyclase through a combination of mRNA display and deep learning

Guthrie Stroh, Douglas Mitchell

Lasso peptides (LasPs) are an intriguing class of natural products that have attracted great interest for their diverse and medicinally-relevant properties, including antibiotic and anti-cancer activity. LasPs are enzymatically cyclized into their eponymous slip-knot fold, which confers exceptional thermal and protease stability. Therefore, LasPs show promise as a topological solution for the challenges in designing orally-available peptide pharmaceuticals. However, the precise mechanism by which this cyclization occurs remains elusive. The research presented here brings newfound insight into the LasP folding process by comprehensively evaluating the substrate fitness of over one billion unique LasP sequences with a promiscuous LasP cyclase. Using a high throughput mRNA display workflow coupled with deep learning neural networks, we have trained a model capable of predicting the ability of any peptide sequence to be folded into a LasP with exceptional accuracy. This work, therefore, paves the way for rational engineering of novel bioactive compounds possessing the therapeutically-desirable properties of LasPs.

24. Biosensor Meets Organ Chip: Cracking the Code of Preterm Birth Inflammation

Hannah Richards

Preterm birth (PTB) remains a global health challenge, with premature preterm rupture of membranes (PPROM) poorly understood, treatments limited, and biomarkers elusive. Current research is hindered by the lack of physiologically relevant models and sensitive diagnostics. We introduce a dynamic duo: an instrumented fetal membrane on-a-chip (FMOC), a 3D microfluidic model of the maternal-fetal interface, paired with a magnetic bead electrochemical sandwich assay (MBESA) biosensor for detecting inflammatory cytokines like IL-1β, pivotal in PTB. The MBESA offers a highly sensitive alternative to traditional ELISAs, targeting inflammation triggered by Group B Streptococcus (GBS), a key PPROM trigger. Our results show that the MBESA reliably detects IL-1β (10–600 pg/mL) in infected FMOC cultures and LPS-treated THP-1 macrophages, with comparable results to traditional ELISA methods. This FMOC-MBESA duo provides a robust, translational tool for studying infection-driven inflammation and complex PTB mechanisms. Future efforts will focus on detecting additional inflammatory cytokines, such as IL-10, and optimizing MBESA performance for organ on-a-chip systems, paving the way for innovative PTB prevention strategies.

25. Virtual Bubbles, Real Insights: A Novel Approach to Mapping Peripersonal Space in Autism and Beyond

Hari Srinivasan, Carissa J Cascio, Mark T Wallace

Peripersonal Space (PPS) is a dynamic sensory-motor interface critical for adaptive functions such as spatial navigation, motor planning, body schema awareness, and social engagement. Alterations in PPS processing have been linked to sensory-motor challenges in autism, affecting obstacle avoidance, object manipulation, and dynamic task recalibration. Understanding PPS in autism is crucial for addressing these challenges and developing tailored interventions. Our novel Bubble Pop task enhances PPS measurement by bridging temporal and spatial aspects, integrating elements of simultaneity judgment (SJ) tasks with reaching-based paradigms to dynamically capture multisensory integration (MSI). While traditional SJ tasks emphasize temporal precision, our approach extends this by incorporating spatial coordination, providing a more comprehensive assessment of PPS flexibility and its neural and physiological underpinnings. Our task is both ecologically valid and designed to be simple to allow the inclusion of a broader profile of autistic participants. To investigate PPS mechanisms, we leverage VR/AR, motion capture, eye tracking, physiological monitoring, and neuroimaging to examine how sensory-motor integration shapes spatial perception and interaction. Preliminary pilot testing in a VR-based version demonstrates feasibility, and anticipated results include a detailed characterization of PPS variability in autism, offering insights into its role in adaptive responses. By linking PPS mechanisms to sensory-motor integration and functional outcomes, this research informs interventions aimed at enhancing spatial navigation, social communication, and sensory-motor coordination in autism. Beyond autism, our novel bubble pop task has broader applications for studying sensory-motor integration in the general population, with implications for neurorehabilitation and human-computer interaction.

26. Targeting Metabotropic Glutamate Receptor 7 to Correct Cognitive Phenotypes in Neurofibromatosis Type 1

Harrison Parent, E. Harriott, S. Chenareddy, A. Taylor, N.M. Fisher, B.J. Stansley, R.R. Lavieri, J.K. Shirey-Rice, J.M. Pulley, R.G. Gogliotti, L. Cutting, Colleen Niswender

Metabotropic glutamate receptor 7 (mGlu7) is a group III metabotropic glutamate (mGlu) receptor regulating glutamate and GABA release which is essential for various learning and memory processes1,2. mGlu7 dysfunction is associated with neurodevelopmental and cognitive impairments including ADHD, seizures, and developmental delay3. A Phenome-wide association study (PheWAS) conducted through the Vanderbilt Institute for Clinical and Translational Research (VICTR) detected a novel association between an expression-attenuating single nucleotide polymorphism (SNP) in the GRM7 gene (rs9870680) and Neurofibromatosis Type 1 (NF1). NF1 is a neurodevelopmental disorder characterized by skin pigmentation abnormalities, benign tumor formation, and cancer predisposition. However, nearly 80% of patients suffer from cognitive impairments in the domains of working memory, visuospatial skills, attention, and reading4,5. Preclinical9,10 and clinical findings11,12 suggest that NF1-related cognitive impairments are driven by excessive GABAergic signaling, and the cognitive profiles of NF1 patients and patients with impaired mGlu7 function have considerable overlap. However, there are no currently approved therapies to treat NF1-related cognitive symptoms, and no currently approved mGlu7-targeting drugs. Due to the associations of mGlu7 with learning deficits and GABAergic regulation, we hypothesize that mGlu7 acts as a modifier and potential therapeutic target for cognitive phenotypes in the NF1 population. To test this hypothesis, we are utilizing a combination of in vitro, ex vivo, and in vivo studies, along with human subjects' studies. This multifaceted approach will enhance our understanding of the correlation between mGlu7 and NF1, establish a molecular mechanism of interaction between these cellular signaling pathways, and evaluate the potential of mGlu7 as a therapeutic target for NF1-associated cognitive symptoms.

27. Type III (VX-445) vs. Type IV (IDOR-4) CFTR Correction and N1303K Small-molecule Screen

Jack Olson

Cystic fibrosis (CF) is a lethal genetic disorder affecting approximately 105,000 individuals globally. It is caused by various loss-of-function mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes a chloride and bicarbonate channel expressed on the surface of secretory epithelia necessary for fluid homeostasis. While approved CFTR modulators (correctors and potentiators) have dramatically improved outcomes for patients with common mutations like

F508del, their efficacy remains limited for the ~10% of patients carrying rare or uncharacterized variants, many of which exhibit distinct pharmacological profiles ("theratypes"). To address this challenge, we combined deep mutational scanning (DMS) with western blot-based trafficking assays to evaluate the effectiveness of two structurally distinct correctors: the approved type III modulator VX-445 and the investigational type IV corrector IDOR-4. DMS enabled the functional profiling of 231 CFTR variants, from which we selected a subset exhibiting differential response for biochemical validation. Additionally, we established a virtual high-throughput screen using Rosetta to identify novel small-molecules targeting N1303K, a mutation that remains poorly responsive to all FDA-approved corrector therapies. These investigations aim to expand the list of treatable CF genotypes and provide new tools to optimize the targeting of CF drugs.

28. Directed evolution of pyridine macrocycles for therapeutic development

Jacob Zuckerman, Hamada Saad, Douglas Mitchell

There is a growing need for therapeutic scaffolds capable of modulating challenging drug targets. While small molecules and biologics dominate current drug discovery, peptides offer a unique middle ground and can engage shallow or extended surfaces often inaccessible to conventional modalities. Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a class of natural products that usually feature macrocyclic scaffolds possessing favorable pharmacological properties, making them ideal scaffolds to be used to develop new therapeutics. My project centers on the pyritides, a recently discovered natural product subclass characterized by a macrocycle constrained by a central pyridine ring. Our lab has identified a biosynthetic enzyme from this pathway, MroD, that accepts highly sequence-diverse substrates, enabling rapid generation of novel macrocyclic peptides. I have developed an enzyme coupled directed evolution workflow using mRNA display that combines a chemical dehydrothiolation step to form dehydroalanines from cysteine residues that allows for MroD to act, allowing for the generation and screening of millions of pyritide variants. I will leverage this system to better understand the complex substrate scope of MroD and identify novel pyritide ligands for a therapeutically relevant target.

29. Decoding the selective chemical modulation of CYP3A4

Jingheng Wang

Drug-drug interactions associate with concurrent uses of multiple medications. Cytochrome P450 (CYP) 3A4 metabolizes a large portion of marketed drugs. To maintain the efficacy of drugs metabolized by CYP3A4, pan-CYP3A inhibitors such as ritonavir are often co-administered. Although selective CYP3A4 inhibitors have greater therapeutic benefits as they avoid inhibiting unintended CYPs and undesirable clinical consequences, the high homology between CYP3A4 and CYP3A5 has hampered the development of such selective inhibitors. Here, we report a series of selective CYP3A4 inhibitors with scaffolds identified by high-throughput screening. Structural, functional, and computational analyses reveal that the differential C-terminal loop conformations and two distinct ligand binding surfaces disfavor the binding of selective CYP3A4 inhibitors to CYP3A5. Structure-guided design of compounds validates the model and yields analogs that are selective for CYP3A4 versus other major CYPs. These findings demonstrate the feasibility to selectively inhibit CYP3A4 and provide guidance for designing better CYP3A4 selective inhibitors.

30. Enhanced Time-Resolved Interactome Profiling to Resolve Secretory Protein Quality Control Dynamics

Jake N. Hermanson, Lea Barny, Lars Plate

Time-resolved interactome Profiling (TRIP) is a method previously developed by our group to determine the sequential order of protein-protein interactions, specifically focused on protein folding, assembly, and trafficking processes in the cell. TRIP relies on a short labeling pulse with the non-canonical amino acid homopropargyl glycine (HPG) to track a newly synthesized client protein population, allowing for Click chemistry derivatization with an affinity handle. Combined with affinity purification-mass spectrometry (AP-MS) and TMT-based quantification, this approach enables the sequential order of protein-protein interactions in an unbiased manner with a client protein of interest. However, the integration of HPG requires an extended labeling time, which ultimately limits the temporal resolution that can be achieved to distinguish protein interactors. We show here that the HaloTag (HT), a bioengineered self-labeling enzyme, addresses this limitation of temporal resolution. HT catalyzes a rapid, irreversible reaction with chloroalkanes, which can be modified with various functional groups. Following a short labeling pulse with blocking from non-functionalized ligands allows for the selective biotin functionalization of a newly synthesized client protein of interest. We focus our investigation on Thyroglobulin (Tg), a secreted thyroid prohormone with many known missense mutations that give rise to protein misfolding and secretion defects. Tg is suitable for methodological refinement due to its slow folding kinetics—it takes approximately one hour to fold completely. Understanding disruptions in coordination with factors involved in folding, trafficking, and degradation could provide insights into cellular mismanagement that limits the correct protein quality control processing of Tg misfolding mutants. Furthermore, we are interested in revealing how disulfide-bond formation is coordinated with other protein quality control processes, such as N-linked glycosylation and lectin-assisted folding. Overall, our approach has the potential to provide deeper insights into protein quality control dynamics in secreted and membrane protein variants that give rise to diverse protein misfolding diseases.

31. Identification of G6PC1 Inhibitors

Karin J. Bosma, Kwangho Kim, Derek P. Claxton, Benjamin P. Brown and Richard M. O'Brien

Three glucose-6-phosphatase catalytic subunits, that hydrolyze glucose-6-phosphate (G6P) to glucose and inorganic phosphate, have been identified, designated G6PC1-3. G6PC1 regulates hepatic glucose production and fasting blood glucose (FBG) levels. Elevated FBG in the normal range has been associated with multiple adverse clinical outcomes, including increased risk for type 2 diabetes and various cancers. In addition, in individuals with type 1 or type 2 diabetes, G6PC1 expression is increased contributing to elevated FBG, a driver of diabetic complications. Therefore, G6PC1 inhibitors that lower FBG may be of prophylactic value for the prevention of multiple conditions as well as an adjunct therapy in individuals with diabetes. The studies described here characterize our progress towards the generation of a partial, allosteric G6PC1 inhibitor, a requirement because complete G6PC1 inhibition leads to glycogen storage disease type 1a. We have found that VU0945627, previously designated by Millenium Pharmaceuticals as Compound 3, is a mixed G6PC1 inhibitor, increasing the Km but reducing the Vmax for G6P hydrolysis. Using this inhibitor as a template in combination with an AlphaFold2-derived G6PC1 structural model and MD simulations, we generated a list of additional, candidate G6PC1 inhibitors. One compound, designated Compound 12, selectively lowers the Vmax for G6P hydrolysis without affecting the Km. Derivatives of this compound will be analyzed in future experiments. These studies will take advantage of our recent discovery of a method to solubilize and purify G6PC1 and differential assays using intact and permeabilized microsomes to ensure that cross inhibition of the G6P transporter, SLC37A4, is avoided.

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33. Isolation and investigation of spirocyclic β-lactone containing oxazolomycins as spliceosome modulators

Kathryn Penton, Sydney Bates, Hannah Thirman, Crissey Cameron, Madeline J. Grider-Hayes, Lars Plate, Jonathan Irish, Brian Bachmann

Spirocyclic β-lactones (SβLs) are ring-strain activated natural products produced by actinomycetota, possessing potent and selective cytotoxicity, and with an unknown pharmacological mechanism. To further the understanding of their mechanism of action and the discovery of new SβLs, we identified a cryptic S□L-producing gene cluster in Streptomyces platensis. Utilizing single cell Multiplexed Activity Metabolomics (MAM) to assess a panel of regulated cell injury and cell death markers in metabolomic arrays tested against MV-4-11 cells, phosphoprotein-S6 (p-S6) phosphorylation inhibition was observed for several metabolomic features, prompting us to prioritize these metabolites for structure elucidation. Several SβL compounds were isolated including one new variant, permitting correlation of a cryptic biosynthetic gene cluster entry for oxazolomycin D (OxD) family metabolites. Cytotoxicity and flow cytometric structure activity relationships for isolated analogs were elucidated using an expanded phospho-flow metabolism marker panel suggesting that SβLs possess two pharmacophores: a mixed polyketide component, involved in target engagement and inhibition, and a nonribosomal peptide synthetase-derived covalent □-lactone warhead, contributing to cytotoxicity. Cellular thermal proteome profiling analysis of oxazolomycin D treated MV-4-11 cells implicated potent modulation of spliceosome associated proteins. Spliceosome-associated functional changes were validated by alternate splicing analysis upon induction by oxazolomycin D. As aberrant RNA splicing occurs in nearly all cancer types, novel spliceosome-associated cellular response profiles of OxD define an unexplored opportunity for therapeutic development in cancers dependent upon alternative splicing.

34. Structural insights on the binding affinity and selectivity of bioisosteric BRD inhibitors

Kesavan Babu, Tim Stachowski, Sourav Das, Priyanka Samanta, Chun-Ju Tsou, Stanley Nithianantham, William Pomerantz, Anang Shelat, Marcus Fischer

Bromodomains (BRDs) are evolutionarily conserved recognition motifs of acetylation marks and key regulators of transcription by recruiting proteins to the transcriptional machinery. BRD proteins are broadly categorized into bromodomain-extra terminal (BET) and non-BET bromodomain containing proteins. BPTF is a non-BET protein that is amplified in pediatric cancers including neuroblastoma, medulloblastoma and osteosarcoma, thereby emerging as a cancer treatment modality. A previously designed inhibitor, BZ1, has several liabilities, including off-target effects on other BRD proteins. BZ2, a BZ1 bioisostere, shows 5000-fold increase in the selectivity of BPTF bromodomain compared to BET-protein BRD9. To understand the affinity differences of these two compounds, we determined a set of high-resolution crystal structures of BPTF-BZ1, BPTF-BZ2, BRD9-BZ1 and BRD9-BZ2 with resolutions of 1.35 Å, 1.95 Å, 1.05 Å, and 1.15 Å, respectively. While the co-crystal structures share similar interactions in that pyridazinone core ring, we observe several differences in the position and interactions of the ligand's aromatic ring and amine tail, and a water-mediated pi-interaction at the aromatic ring of BZ2 that is lost when bound to BRD9. Together these differences provide insights into the reduced affinity of BZ2 to BRD9 compared to BZ1. In addition, sequence differences in the binding pocket of BRDs recapitulate differences in binding affinity and selectivity of the BZ compounds that can be utilized in designing selective inhibitors.

35. Development of the first vascular-specific KATP channel inhibitors for treating disorders of vascular function

KJ Li, Craig W. Lindsley, Elaine L. Shelton, Alican Gulsevin, Jian Gao, Colin G. Nichols, Jerod S. Denton

The ductus arteriosus (DA) is an essential fetal structure that shunts blood away from the high-resistance pulmonary circulation and toward the placental circulation, where fetal-maternal gas exchange occurs. Normally, after birth, the DA undergoes vasoconstriction and closure in response to increased arterial blood oxygen tension and decreased prostaglandin levels. The failure of the DA to close, or patent DA (PDA), is one of the most common congenital heart disorders in newborns, and therapeutic options are limited to non-specific medications that target prostaglandin pathways. ATP-regulated inward rectifier potassium (KATP) channels comprising Kir6.1 and SUR2B subunits are enriched in the DA and negatively regulate vascular tone, suggesting that inhibitors will induce vasoconstriction and DA closure. Testing this hypothesis will require the development of highly specific inhibitors that can discriminate between Kir6.1/SUR2B and distinct KATP channel subtypes (e.g., Kir6.2/SUR1) expressed in the pancreas, brain, and other organs. We therefore performed a high-throughput screen of 47,872 compounds for novel modulators of Kir6.1/SUR2B. The most potent inhibitor discovered is VU0542270, which inhibits Kir6.1/SUR2 with an IC50 of approximately 40 nM and is highly selective for Kir6.1/SUR2B over Kir6.2/SUR1 and several other Kir channels. In silico docking and functional characterization of putative binding site mutants suggest that VU0542270 and glibenclamide interact with distinct yet partially overlapping binding sites in SUR2. We also present the structures of four new SUR2-specific inhibitors and anticipate that these studies will inform the next-generation vascular KATP channel inhibitor design.

36. Developing Highly Selective Intracellular Binding Dopamine D2 Receptor Antagonists

Kole Martin, Takudzwa Mabvuta, Rajani Nimma, Salisha Baranwal, Edwin Matthiessen, Prashant Donthamsetti

Drugs serve as the cornerstone of modern medicine by allowing us to control the activity of specific molecules in the body. However, existing drugs have substantial limitations that lower their effectiveness. Conventional drugs (small molecules, peptides/proteins) can be dose adjusted and are reversible, but they bind off-target molecules throughout the body. Alternatively, gene therapy can be used to alter the target protein with molecular and spatial specificity, but these alterations cannot be dose adjusted and are permanent. To solve these problems, we are developing a new platform technology called Tag-Guided Drug that combines the doseability and reversibility of conventional drugs with the molecular, cellular, and spatial specificity of gene therapy. Tag-Guided Drug has two components: (1) a small peptidic affinity tag that is inserted in or near a functionally important site in the target protein using gene editing, and (2) an antibody that selectively and reversibly binds the introduced tag. When bound to the tag, the antibody sterically blocks other molecules from binding the target protein and/or induces a conformational change in the target protein that alters its function. In proof-of-concept studies, we applied Tag-Guided Drug to dopamine 2 receptor (D2R), a G protein-coupled receptor (GPCR) that plays key roles in health and disease. We predicted that antibody binding to a tag that is inserted into the intracellular face of D2R would prevent signaling proteins such as G protein from coupling to the receptor. Guided by molecular modeling, we inserted an α-helical ALFA-tag into various intracellular loops (ILs) of D2R. NbALFA, an antibody that binds ALFA-tag with picomolar affinity, inhibited a D2R mutant that contains ALFA-tag in IL3 but not wildtype D2R, indicating that Tag-Guided Drug works. To enhance Tag-Guided Drug performance, we further optimized the positioning of the ALFA-tag in D2R and engineered more potent NbALFA variants. Taken together, our findings serve as a template for developing highly selective Tag-Guided Drugs for diverse protein targets that have historically been difficult-to-target with conventional approaches.

37. High-Throughput Screening Identifies Small Molecule Modulators of KCNQ1 Trafficking

Katherine R. Clowes Moster, Mason C. Wilkinson, Carlos G. Vanoye, Katherine M. Stefanski, Kathryn R. Brewer, Alfred L. George Jr., Charles

Sanders

Approximately 1 in 2500 individuals suffer from congenital long QT syndrome (LQTS), a cardiac disorder that can cause syncope, cardiac arrythmia, and cardiac arrest, which can be fatal. Loss-of-function mutations in the voltage gated potassium channel protein KCNQ1 cause type 1 long QT syndrome (LQT1), which accounts for 30-50% of cases of LQTS. Over 250 disease-associated mutations in KCNQ1 have been identified, but it is unknown whether there is a common mechanism through which these mutations impact KCNQ1. Previous studies have found that mistrafficking may play a role in KCNQ1 dysfunction. Only ~20% of expressed WT KCNQ1 successfully traffics to the plasma membrane, and many LQT1-associated mutations in KCNQ1 decrease trafficking efficiency further. Protein mistrafficking has been identified as a mechanism of several diseases and has been found to be rescuable with small molecules. This led us to hypothesize that mistrafficking is a common mechanism of KCNQ1 loss-of function in LQT1, and that small molecules can alter the trafficking efficiency of KCNQ1. To test this hypothesis, we developed a high content imaging-based trafficking assay to screen for compounds that alter the trafficking of KCNQ1 in mammalian cells. Screening of small molecule libraries identified several hits that alter KCNQ1 expression and/or trafficking. Specifically, one hit compound has been identified that increases cell surface KCNQ1 and leads to an increase in KCNQ1 current in CHO cells. An additional hit compound decreases total and cell surface KCNQ1. This compound has been shown to destabilize KCNQ1 and reduce the expression of disease-linked “super trafficking” KCNQ1 R231C mutant back to WT-like levels. Follow up experiments to determine the mechanism of action of these hits and begin preliminary SAR studies are ongoing. This work contributes to our larger hypothesis that misfolding-induced mistrafficking is a common, rescuable, mechanism of KCNQ1 dysfunction and informs potential routes for treatment of cardiac disorders like LQT1.

38. Mapping the Allosteric Landscape of the μ-Opioid Receptor to Engineer Safer Analgesics
R. Justin Lindsay, N. Kithmini Wijesiri, Tatum Murdock, Benjamin P. Brown

The μ-opioid receptor (μOR) is the primary target for our most potent analgesics and the principal mediator of opioid addiction. A central challenge in developing safer therapeutics is to decouple the receptor's analgesic effects from life-threatening side effects, which we hypothesize requires precise control over downstream signaling. Different ligands can stabilize distinct μOR conformational ensembles, leading to the selective recruitment of specific G-protein subtypes; however, the atomic-level mechanisms that translate a ligand's chemical properties into a specific functional signaling outcome remain poorly understood. To bridge this gap, we are using massive-scale molecular dynamics (MD) simulations, totaling over 1.5 milliseconds and ongoing, coupled with hidden Markov models (HMMs) to construct a comprehensive free energy landscape and kinetic model of the μOR ligand-dependent signaling. This allows us to quantify conformational ensembles and microswitch transition rates, providing a detailed mechanistic explanation for their distinct pharmacological profiles. By simulating the μOR in its apo state and bound to a diverse panel of pharmacologically distinct ligands, our work aims to reveal the unique structural rules governing behavior across the spectrum of agonists, partial agonists, and antagonists. To connect these ligand-specific dynamics to cellular function, we explicitly model the receptor's coupling to its downstream G-protein partners. We first generate conformational ensembles for six relevant Gα subtypes (GαI1, GαI2, GαI3, GαOA, GαOB, and GαZ) via all-atom MD simulations of the corresponding Gαβγ heterotrimers. We then perform large-scale ensemble docking, pairing these G-protein conformers with individual microstates from our μOR free energy landscapes. By statistically reweighting the landscape based on these interactions, we quantify how each bound ligand reshapes the G-protein coupling surface. This yields predictions of G-protein selectivity, establishing a direct mechanistic link from ligand binding to cellular response and providing a structural basis for biased agonism. These detailed mechanistic insights are now enabling large-scale structure-based virtual screening to discover novel ligands with finely-tuned signaling properties, providing a comprehensive platform for engineering next-generation opioid therapeutics.

39. Transition Dipole Strength as a Quantitative Tool for Protein Secondary Structure Analysis
Lindsey Weissman, Amanda Cao, Lauren Buchanan

Proteins adopt complex structures through dynamic folding processes that are challenging to capture experimentally. This work advances our understanding of the structural information that can be obtained by transition dipole strength (TDS) analysis. TDS is an innovative extension of two-dimensional infrared (2D IR) spectroscopy. By systematically characterizing the TDS of model α-helical peptides of varying lengths, we find there to be a linear correlation between TDS values and helical length. This data can then be used to extrapolate the number of residues in the longest α-helix in globular proteins, even when multiple helices are present. This work demonstrates TDS analysis as a promising method for the elucidation of structural dynamics that cannot be obtained by other methods, especially in complex protein architectures, while highlighting the need for an increased understanding of the interplay of higher order structural organization with vibrational delocalization.

40. Optimizing Sortase-Mediated Conjugation of anti-hCOX-2 Nanobodies for Imaging Applications
Lucy Schneider, Jashim Uddin, Michael Goodman, Ansari Aleem, and Lawrence J. Marnett

Cyclooxygenase-2 (COX-2) is an inducible enzyme that catalyzes key steps in the biosynthesis of prostaglandins, playing a central role in inflammation and pain. It is commonly overexpressed in inflamed tissues, making it a valuable biomarker for imaging inflammatory processes. B6 and F7 nanobodies have been shown to bind human COX-2 (hCOX-2) with high specificity and affinity. By conjugating these nanobodies to a fluorescent dye, such as TAMRA, they can be employed to visualize sites of inflammation both in-cellulo and in-vivo. This work focuses on optimizing Sortase A-mediated site-specific conjugation of anti-hCOX-2 nanobodies to TAMRA, with the goal of improving imaging efficiency and preserving nanobody functionality.

41. Iron-sparing response sustains Bacteroides thetaiotaomicron resilience in the inflamed gut
Madeline Bresson

Iron is a nutritional metal essential for nearly all living organisms on the planet, necessary for a wide range of cell functions in the host and bacteria, including DNA replication. Vertebrates have evolved to exploit this dependency by sequestering trace minerals, such as iron, to limit bacterial proliferation during infections, a defense strategy termed nutritional immunity. While pathogens are known to deploy exquisite sets of strategies to overcome iron limitation to colonize the host, how commensal bacteria survive iron limitation during gut inflammation remains poorly understood. Here, I show that Bacteroides thetaiotaomicron (B. theta), an abundant representative of the human gut commensals, sustains resilience by reducing iron expenditure and prioritizing its allocation to essential cellular functions, a process termed iron-sparing response. Specifically, I demonstrate that B. theta upregulates iron-free electron carrier, flavodoxin (fldA), to replace its iron-containing counterpart, ferredoxin, under iron starved conditions. Using in vitro and murine infectious colitis model, I show that iron-sparing is critical for commensal resilience in the inflamed, iron-deprived gut.

42. Direct-to-Biology Enabled Molecular Glue Discovery
Maowei Hu

Molecular glues powerfully control protein proximity but have largely eluded direct screening. A promising avenue for addressing this challenge lies within pinpointing the fundamental features for function-first identification of molecular gluing events. In the widely accepted mechanism, a molecular glue stabilizes two proteins within a ternary complex – here we show how differences in affinity for ternary and binary complexes directly categorize glues from non-glues. We leverage these differences together with high-throughput chemical synthesis and affinity-selection mass-spectrometry to discover a molecular glue degrader from a suite of over 20,000 crude chemical reaction mixtures. Orthogonal assays robustly support identification of molecular glues via ternary complex

stability. Our findings suggest a roadmap for de novo molecular glue discovery lies within kinetic profiling of unpurified mixtures of small organic molecules against protein pairs.

43. Small Molecule Modulation of PMP22 Expression and Trafficking
Mason Wilkinson, Katherine Stefanski, Katherine Clowes, Pramod Gowda, Thomas Hasaka, Emily Days, Josh Bauer, Bruce Carter, Charles Sanders

Mutations in the peripheral myelin protein 22 (PMP22) gene can result in Charcot-Marie-Tooth disease types 1A and 1E (CMT1A and CMT1E), hereditary neuropathy with liability to pressure palsies (HNPP), or Dejerine–Sottas syndrome (DSS). CMT1A and HNPP occur when Schwann cells produce too much or too little PMP22, respectively, which leads to abnormalities in the myelin sheath. We hypothesize that chemical compounds that selectively increase or decrease PMP22 levels in Schwann cells could be applicable to developing treatments for CMT1A, HNPP, and possibly some cases of CMT1E/DSS. Because no FDA-approved treatments currently exist for any of these diseases, we sought to screen for small molecule compounds that can alter PMP22 levels in Schwann cells. To this end, we developed a rat Schwann cell (RSCs) line that can express human PMP22 on demand when we add the antibiotic doxycycline to their growth medium. We first grew these cells at large scale and treated individual populations each with one of 22326 small molecule compounds. Next, we evaluated each compounds effects on Myc-hPMP22 levels at the RSC plasma membrane using high-throughput fluorescence microscopy. Following extensive curation, we have identified three hit compounds that dramatically and specifically alter Myc-PMP22 protein levels without causing cytotoxicity and without perturbing transcription from the exogenous locus. At low micromolar treatment conditions, two compounds largely eliminate Myc-hPMP22 protein levels, while the third nearly doubles the levels measured in untreated cells. We are now pursuing structure-activity relationship experiments to identify more potent chemical analogues of these hits, as well as evaluating their to alter endogenous PMP22 expression and rectify myelination in rat dorsal root ganglion explants.

44. Reimagining the Synthesis of Rearranged Terpenes: Utilizing Borylcarbonylation to Rapidly Access the Core Scaffold of Mitchellene B
Maxwell Hughes, Alexander W. Schuppe

Terpenes are a diverse class of natural products which have been shown to possess a broad range of pharmacological activity, including antimicrobial, anticancer, and anti-inflammatory. These small molecules are efficiently constructed from simple five carbon isoprene units by a myriad of organisms, such as plants, bacteria, fungi, and even some insects. To date, there are over 50,000 known structures with rich stereochemistry and intricate polycyclic architecture, which has fascinated and inspired synthetic chemists for decades. Many chemists have successfully emulated nature by developing radical and cationic polyolefin cyclization pathways to rapidly access the *trans*-6-membered scaffolds observed in many ring-intact terpenes. However, these methods are not suitable for synthesizing rearranged terpenes, which feature more complex ring systems. My goal is to resolve this retrosynthetic gap by developing a hydroboration-carbonylation protocol to stitch olefin precursors into these rearranged polycyclic scaffolds. I plan to utilize this new strategy to achieve the first total synthesis of mitchellene B, a tetracyclic sesquiterpene lactone isolated from the Australian shrub *Eremophila mitchelli* in 2011. Once I have established an optimal synthetic sequence, I plan to collaborate with the Vanderbilt High Throughput Screening Facility to elucidate the biological activity of mitchellene B as well as any derivatives.

45. Thio-oxazole modified peptides biosynthesized by multinuclear iron-dependent oxidative enzymes (MNIOs)
Mayuresh G. Gadgil, Shravan R. Dommaraju, Xiaopeng Liu, Alex J. Battiste, Miriam H. Bregman, Douglas Mitchell

Multinuclear iron-dependent oxidative (MNIO) enzymes are a class of peptide-modifying enzymes involved in the biosynthesis of ribosomally synthesized and post-translationally modified peptide (RiPP) natural products. A recently published report details how one member of this enzyme family transforms Cys residues in precursor peptides bearing -EGKCG- motifs into oxazolone/thioamide moieties. By analyzing closely related homologs of this enzyme which also act on similar substrate motifs, we provide spectroscopic evidence to suggest that the formed post-translation modification is in fact a (5-thio)-oxazole rather than the oxazolone/thioamide structure. Mass spectrometry demonstrated that the (5-thio)-oxazoles effectively bind metals, thereby suggesting the functional utilization of these natural products as defense against metal-induced stress. Currently ongoing work is exploring the role of these gene clusters in evasion from metal-mediated immune responses.

46. Evaluating the role of microRNAs in regulating metabotropic glutamate receptor 7: implications for neurodevelopmental disorders
Genevieve Hunn, Niki Harris, Colleen M. Niswender

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder (NDD) caused by mutations in the Methyl CpG Binding Protein 2 (MECP2) gene. With a prevalence of 1 in 10,000 live female births, children who are affected start regressing in their social, motor, and cognitive abilities around 6 to 18 months of age. While current therapeutic strategies focus on restoring MECP2 levels, there are limited treatments due to the complexity of symptoms and off-target effects. Interestingly, patients diagnosed with RTT show a 60-70% reduction in metabotropic glutamate receptor 7 (mGlu7) protein expression in brain samples. Mouse models of RTT also show reduced mGlu7 expression that results in cognitive deficits. These deficits can be corrected through positive allosteric modulation of mGlu7 activity, but there are also off-target effects. Therefore, using additional strategies to selectively increase mGlu7 receptor expression may be a viable therapeutic approach for RTT. However, the mechanism responsible for reduced mGlu7 expression remains unclear. Recent studies have shown that MeCP2 mutations can regulate the expression of microRNAs (miR) in RTT patients and mice. miRs function by binding to the 3' untranslated region (UTR) of target mRNA to prevent protein translation or target the mRNA for degradation. Therefore, dysregulated miRs may drive the observed MeCP2 deficit. While multiple miRs are predicted to bind to the GRM7 3'UTR, specifically miR-15a was upregulated by ~25-fold and miR-133a was downregulated by ~4-fold in the temporal cortices of RTT patient samples. Interestingly, we have shown that miR-15a results in a concentration-dependent decrease in luciferase activity using a GRM7 3'UTR luciferase reporter construct, which can be reversed with a miR-15a inhibitor. Future experiments include assessing these miRs against the full-length mGlu7 construct and in neuronal cultures, along with designing site-blocking antisense oligonucleotides with phosphorothioate and locked nucleic acid chemical modifications that have optimal inhibitory effects on these miRs. Ultimately, we anticipate this approach will investigate underlying mechanisms driving reduced mGlu7 expression and will expand potential therapeutic strategies for RTT.

47. Sex Differences in metabolic regulation by Gi/o-coupled receptor modulation of exocytosis
Montana Young, Ryan P. Ceddia, David Reyes, Jackson B. Cassada, Analisa Thompson-Gray, Sheila Collins, Heidi E. Hamm

Presynaptic Gi/o coupled GPCRs can act as negative feedback regulators of neurotransmitter release through two mechanisms via Gβγ effector modulation: decreased calcium influx and direct inhibition of membrane fusion by soluble N-ethylmaleimide– sensitive factor attachment protein (SNAP) receptor (SNARE). Previously, we discovered that truncation of the last three C-terminal amino acids of SNAP25 (SNAP25Δ3) prevents Gβγ-SNARE interaction effectively removing the braking mechanism on neurotransmitter release. We have demonstrated enhanced metabolic protection in male SNAP25Δ3 mice housed at room temperature (22^{o}C) including increased adipose tissue beiging and glucose uptake and enhanced insulin sensitivity, rendering them resistant to diet-induced obesity (DIO). When male SNAP25Δ3 mice were housed at thermoneutrality (30^{o}C) all metabolic protection was abolished, suggesting sympathetic tone is important for the phenotypes. Here, we find SNAP25Δ3 female mice have the same metabolic protection at RT (22^{o}C), and also displayed enhanced metabolic protection from DIO compared to standard chow. However, female SNAP25Δ3 mice display persistent metabolic protection even when housed at thermoneutrality. In this study, we investigate the mechanisms behind this sex dependent phenotype

persistence. Thermoneutral set point did not differ between sexes nor genotype suggesting this effect is not due to a hypothalamic temperature regulation difference. Ovariectomizing the SNAP25Δ3 mice did cause weight gain, but these mice still displayed enhanced metabolic protection compared to the ovariectomized wild-type mice. Therefore, we hypothesize there is a sex hormone independent mechanism driving the persistent metabolic protection of female SNAP25Δ3 animals housed in thermoneutrality that is not present in the male SNAP25Δ3 mice.

48. Compound Management Center: Automated Sample Management and Small Molecule Library Reformatting
Mariana Santana Smith

The Compound Management (CM) center under the Department of Chemical Biology & Therapeutics at St. Jude Children's Research Hospital has a long history of facilitating small molecule screening campaigns across the institution. Since 2005, CM has continuously expanded a diverse inventory of over 800,000 unique small molecules sourced from commercially available libraries. The CM library is audited using the Titian Mosaic Sample Management Inventory software, and is maintained using various automated stores at -20 ℃. In 2017, CM began updating the lab's automation capabilities to meet industry standards. This included the acquisition of multiple liquid handling devices, as well as the commissioning of an automation platform that integrates a precise robotic arm with various benchtop devices. As a result, CM can expediate the primary screening, hit finding, and lead optimization steps of the drug discovery process by making small molecule compounds readily accessible in the following formats: powder/liquid aliquots, custom single-point and dose response plates, compound library plates, and custom assay-ready plates.

49. The Role of Synonymous Mutations on Proteostasis and Cellular Interactions During Viral Host Adaptation
Molly E. Sullivan, Paul Azzinaro, Gisselle N. Medina, Lars Plate

RNA viruses mutate rapidly, facilitating their adaptation to diverse environments and increasing the need for effective therapies against RNA viruses to prevent 'spillover' events between host species. One promising strategy involves incorporating synonymous mutations into viral genomes to attenuate RNA viruses and develop live-attenuated vaccine candidates. This approach leverages the disruption of codon bias to significantly reduce viral fitness while producing a host immune response resembling that of the virulent strain. While synonymous mutations do not alter the amino acid sequence of the encoded protein, recent studies have shown that they influence several cellular processes, such as mRNA structure and folding, mRNA splicing and stability, and co-translational folding. Here, we use foot-and-mouth disease virus (FMDV) as a model to study the effects of synonymous mutations on cellular processes. Deoptimized synonymous mutations have been introduced into the P1 capsid coding region of the FMDV genome to determine the interplay between translational rates, viral protein folding, and interactions with critical cellular machinery. Several differing protein-protein interactions have been identified between wild-type and deoptimized P1 in human cells, including protein homeostasis (proteostasis) factors. Additionally, synonymous mutations have been shown to result in less efficient capsid processing by the viral protease 3Cpro. Characterization of how these interactions influence critical cellular processes, such as protein folding and viral capsid assembly, will provide insights into the function of synonymous mutations during viral host adaptation.

50. Adaptive Evolution of the Obligate Anaerobe Bacteroides thetaiotaomicron Reveals Key Protein Implicated in Oxygen Tolerance
Muen Shen

At homeostasis, the mammalian gastrointestinal tract is anaerobic and inhabited by trillions of microorganisms, collectively termed the gut microbiota, which play critical roles in host physiology. Most bacteria in the healthy gut are obligate anaerobes, organisms that cannot grow in the presence of oxygen. During gut inflammation, levels of oxygen and reactive oxygen species (ROS) increase substantially, constituting lethal challenges to anaerobes. While oxygen plays a significant role in shaping the composition and functions of the microbiome, how it impedes anaerobe growth remains poorly understood. To investigate this, I generated a strain of the obligate anaerobe Bacteroides thetaiotaomicron (B. theta) capable of growing in oxygenated conditions through adaptive evolution. Whole genome sequencing analysis identified a mutation (C222Y) in an uncharacterized protein, previously termed Oxe. Structural modeling revealed its homology to flavodiiron proteins known to catalyze the reduction of O2 to H2O and/or NO to N2O. Interestingly, knocking out oxe confers a significant fitness advantage in hypoxic conditions, enabling robust growth under 3% O2. Complementing the Δoxe strain with wild-type oxe—but not the mutant oxe—restored oxygen-sensitivity. Based on these observations, we hypothesize that Oxe reduces O2 to H2O2. Indeed, Δoxe exhibits significantly less H2O2 generation compared to wild-type B. theta when exposed to room air (21% O2). Together, these findings suggest Oxe is an important contributor to the growth barrier of anaerobes under oxic conditions, advancing our understanding of microbial adaptation to oxygen and paves the way for potentially developing oxygen-tolerant obligate anaerobes for probiotics applications.

51. Nanobodies against *Clostridioides difficile* CDTb provide a toolkit for toxin neutralization and quantitation
Kateryna Nabukhotna, David M. Anderson, Maria McGresham, John A. Shupe, Ruben Cano Rodriguez, Rebecca A. Shrem, Brian Wadzinski, Benjamin W. Spiller, Borden Lacy

Clostridioides difficile is a pathogenic bacterium and a leading cause of antibiotic-associated life-threatening diarrhea in the United States. The symptoms of the infection arise due to production of toxin A and toxin B. These toxins disrupt the intestinal barrier and cause an acute host inflammatory response, a major hallmark of pathophysiology in C. difficile infection (CDI). Epidemic C. difficile strains additionally produce the C. difficile transferase toxin (CDT, a binary toxin consisting of catalytic CDTa and delivery CDTb) suggesting that it may be important for the severity of CDI. However, the role of CDT during C. difficile pathogenesis remains poorly understood, partially due to limited research tools for inhibiting toxin activity and knowing when the toxin is present. We created a CDTb-specific nanobody clone library and chose to express and purify five with promising CDTb-binding properties. Studies using the Carterra LSAXT platform revealed high affinity binding interactions with three distinct CDTb epitope groups. We tested the nanobodies from two different epitope bins as capture-detection pairs for their capacity to function in a sandwich ELISA assay. This assay enabled us to quantify CDTb produced in the cecum of mice over the time course of an in vivo infection. Additionally, we found that these nanobodies had potent neutralizing activities that protected epithelial Vero-GFP cells from CDT cytopathic effects. AlphaFold3 structure predictions allowed us to map neutralizing epitopes on the toxin to hypothesize mechanisms of toxin neutralization. We are working on developing in vitro functional assays to confirm predicted mechanisms by which nanobodies neutralize toxin activity, for example via blocking of CDTb oligomerization and assembly necessary for the delivery of catalytic CDTa into cell cytosol. We anticipate that these reagents will allow researchers to expand toxin intervention and monitoring strategies needed to obtain a deeper understanding of the CDT mechanism of action during CDI.

52. BRAID; A Better Toolkit for Combination Analysis
Nathaniel R. Twarog, Ayush Attery, Anang A. Shelat

Popular combination evaluation methods like Bliss volume and its close-related variant ZIP, though widely used and simple to implement, exhibit pronounced biases driven by dose-response curve shape that produce stereotypical patterns of supposed "interaction". Using publicly available datasets and machine learning, we show that these patterns can be easily learned and can even be predicted from individual compound behaviors alone. These biases, along with instabilities arising from non-linear error propagation, result in methods which carry considerably less mechanistic information than methods build on response surface models, such as BRAID or MuSyC, demonstrated in a pan-method evaluation of the Merck Oncopolypharmacology screen. The robustness of BRAID, combined with a more holistic evaluation of combination behavior, enables us to identify pronounced cell-line-dependent interactions in a high-throughput screen of anticancer drugs in rhabdoid tumors. Additionally, by adjusting the mathematical application of BRAID, we can model less-traditional atypical

interactions, such as oppositional or protective surface in which agents produce contrary effects. We use these extended BRAID models to quantify and compare highly varied response surfaces in a high throughput screen of antifungal drug combinations. In conclusion, a response surface method of combination evaluation, such as BRAID, provides an unbiased, more robust, and more flexible approach to understanding drug combinations, enabling greater insight into mechanistic behaviors and more actionable predictions in therapeutic development.

53. Discovering Blood-Based Biomarkers for Hypertension Using Quantitative Proteomics

Nekesa C. Oliver, Khiry L. Patterson, Julia B. Libby, Liping Du, Kun Bai, Yajing Li, Timothy J. Hohman, Renã A. S. Robinson

Hypertension (HTN) is a critical health condition that affects nearly 50% of Americans aged 35 and older, with African-American adults commonly reported to have the highest prevalence in the world1,2. The molecular profile of HTN is complex and highly heterogenous and although inflammation, the renin-angiotensin-aldosterone system (RAAS), salt sensitivity, and endothelial dysfunction have been identified as key pathways, the mechanisms that lead to increased prevalence in African American adults are unclear. This study utilizes quantitative proteomics, to establish a proteomic profile of HTN in an exclusively African American cohort from the Southern Community Cohort Study (SCCS). High-confidence LC-MS/MS data was acquired for plasma samples from SCCS (N = 808) multiplexed into 58 TMTpro 16-plex batches. Quality control (QC) metrics from an external QCpool sample guided acquisition and robustness of data on an Orbitrap Fusion Lumos instrument. At the halfway point of data acquisition, MS data were normalized across 29 batches and preliminary results identified 2710 proteins present in 50% of participants (N = 406 samples). Canonical HTN-related genes such as Angiotensin-converting enzyme (ACE), Alpha-Adducin 1 (ADD1), and renin (REN) were detected however had no change in expression thus far in samples from normotensive and hypertensive adults. Linear regression models that adjusted for age and sex were applied and 23 differentially-expressed proteins were statistically significant (adjusted p <0.05) between normotensive and hypertensive samples. Notably of these, Afamin (AFM), known for its role in oxidative stress regulation, was differentially-expressed which is consistent with recent findings in a South African cohort. STRING pathway analysis with k-means clustering of differentially-expressed proteins revealed a cluster with significant association (p <0.05) to amyloidogenic processes, pertinent to cardiovascular pathologies. These preliminary findings represent a pivotal step toward elucidating the molecular underpinnings of HTN and understanding disparities in hypertension in African American adults.

54. Exploring Environmental Influences on Actinomycete Diversity and Secondary Metabolism in Hypogean Ecosystems

Paul Kastner, Brian O. Bachmann

There is an urgent need for new antibiotics and cancer treatments. Natural products have historically proven successful, accounting for nearly 70% of small molecule drugs discovered in the past four decades. One particularly productive source of natural products, the phylum Actinomycetota, has facilitated the discovery of over 90% of commercially available antibiotics. However, the discovery rate of novel bioactive molecules has slowed. A driving factor behind this decline is that prominent sources of microbial natural products like the genus Streptomyces have been mined extensively, resulting in the increasingly common rediscovery of known bioactive molecules. To date, most Streptomyces drug discovery has relied upon soil microbiota. Generally, unique and understudied environments with increased biodiversity are also sites of increased chemical diversity. One promising example of a unique and understudied ecosystem for microbial chemical ecology is caves. Examples of the unique properties of caves include their aphotic (lightless) and oligotrophic (nutrient-poor) nature, as well as the relative geographical isolation of these ancient structures. To date, the aptitude of caves as a potential source of microbes that produce secondary metabolites remains unknown. We hypothesize that conditions within hypogean (cave) environments support unique Actinomycetota and their corresponding secondary metabolism in comparison to equivalent epigean (surface) environments. We propose to use a multipronged approach utilizing metagenomics, in situ and ex situ "culturomics", strain isolation, and bioinformatics characterization of secondary metabolism to determine the degree to which hypogean environments host microbes with unique secondary metabolic potential.

55. RIG-I-Activating Nanoparticles Enhance Anticancer Immune Responses

Payton Stone, John T. Wilson

Cancer immunotherapy has revolutionized the fields of oncology and drug delivery, with immune checkpoint blockade (ICB) demonstrating remarkable potential as an alternative to traditional treatment regimens. Although ICB has demonstrated disease control in certain cancers, it has also indicated limited therapeutic efficacy in poorly immunogenic cancers. Through the activation of specific cellular pattern recognition receptors (PRRs), the immunosuppressive tumor microenvironment (TME) of these cancers can be reprogrammed to a more immunogenic, 'hot' phenotype with larger populations of infiltrating T cells, directly correlating with improved responses to ICB. Indeed, activation of the retinoic acid-inducible gene I (RIG-I) pathway has been found to elicit a downstream signaling cascade resulting in the production of type-I interferons which can induce this shift in the TME. Unfortunately, most RIG-I-activating therapeutics are not able to freely cross the cell membrane where they are required to activate this cytosolic PRR. In this work, we demonstrate the ability of 3pRNA cargos to be loaded within pH-responsive, endosomolytic polymeric nanoparticles by use of a flash nanoprecipitation (FNP) fabrication method. We next analyzed our library of RIG-I activating nanocarriers (RANs) for physical properties and determined all formulations to be vesicular and uniform (PDI < 0.3) in nature, with encapsulation efficiencies (EE) of ~50%. All RAN formulations were then further analyzed for endosomolytic activity, surface charge, morphology, and in vitro activity in both primary cells and reporter cells. To elucidate in vivo behavior, we fluorescently labeled RANs and tracked their retention at the tumor site upon intratumoral administration and biodistribution upon systemic administration. Finally, we intratumorally treated murine colon cancer and melanoma models with RANs and assessed the ability of the formulation to mitigate disease progression and prolong survival. Collectively, this work demonstrates that FNP can be harnessed as a versatile and scalable process for efficient loading of nucleic acids into polymeric nanoparticles and highlights the potential of RANs as a translationally promising platform for cancer immunotherapies.

56. EnzyHTP: High-throughput enzyme modeling platform enables new ways to understand and engineer enzymes

Qianzhen Shao, Chris Jurich, Xinchun Ran, Yaoyukun Jiang, Yinjie Zhong, Ning Ding , Zhongyue J. Yang

Enzyme modeling, such as molecular mechanics (MM), quantum mechanics (QM), and multiscale QM/MM modeling, have been extensively utilized to gain insights into enzyme catalysis and develop novel enzymes. However, to perform enzyme simulations, one needs to learn around 6-10 different software from constructing models to post-analysis and manually configuring, debugging, and reformatting input/output files. This renders enzyme modeling highly specialized and labor-consuming, making it challenging to model enzymes and their variants on a high-throughput scale and in a reproducible manner.

In this poster, I will present how we addressed this challenge by developing EnzyHTP, a high-throughput platform that automates the complete life cycle of enzyme modeling and applying EnzyHTP in real applications that engineer enzymes and understand the catalytic origin of enzymes. The core of the EnzyHTP platform is an open-source Python library, and a web interface featuring an AI-agent-based virtual collaborator. While the Python library allows full control of the modeling workflow, it requires some coding expertise. As compensation, the web interface, EnzyHTP-GPT, targets out-of-field users such as experimental scientists. EnzyHTP-GPT minimizes the learning barrier and technicality by leveraging AI-agents based on the Large Language Model (LLM, e.g.: ChatGPT).

The EnzyHTP platform enables new ways to understand and engineer enzymes. We investigated how the non-electrostatic component of substrate positioning dynamics (SPD) affects transition state (TS) stabilization of enzyme catalysis. Using high-throughput enzyme modeling and experimental kinetic assay, we observed a valley-shaped, two-segment linear correlation between the TS stabilization free energy and

substrate positioning dynamics, indicating the substantial contribution of the non-electrostatic component of SPD to enzyme catalytic efficiency. We trained a chirality-aware deep learning model, EnzyKR, to predict outcomes of the hydrolase-catalyzed kinetic resolution. With the encoded enzyme-substrate complex as part of the, EnzyKR outperforms the state-of-the-art model in 18 out of 28 reactions, occupying 64% of the test cases. We developed a physics-guided computational tool, SubTuner, for modifying enzymatic substrate preference. SubTuner is tested with three engineering targets in two different enzymes. All the tests demonstrate SubTuner's ability to accelerate the discovery of function-enhancing mutants for non-native substrates. Overall, EnzyHTP and the high-throughput workflows enabled by EnzyHTP will pave the way for the future of enzyme engineering.

57. A total synthesis of feglymycin enabled by a heterocyclic organocatalyst and Umpolung Amide Synthesis

Preston C. Gourville, Jade A. Bing, Rashanique D. Quarels, Sergey V. Tsukanov, Kenneth E. Schwieter, Kazuyuki Tokumaru, Amanda B. Stephens, Dawn M. Makley, Bo Shen, Abigail N. Smith, Jeffrey Johnston

Aryl glycinamides are a common motif in natural products with antiviral and antibacterial properties. Strategies to access these non-canonical residues have relied on asymmetric methods such as the Sharpless aminohydroxylation of styrenes, and multistep synthesis targeting the desired amino acids that can be coupled using condensative coupling reagents. This approach presents several downsides, including the risk of epimerization of α-stereocenters, hazardous reagents, and the stoichiometric use of coupling reagents with high molecular weights. Shifting the paradigm of carboxylic acids and coupling reagents to Umpolung amide Synthesis (UmAS) brings together a halonitroalkane and N-halamine to forge amide bonds without epimerization risk. Furthermore, the non-condensative nature of UmAS requires a distinct complement of reagents with a less hazardous profile. However, UmAS has been untested on mid-sized peptides until now. This work will describe how asymmetric aza-Henry reactions with quinoline-based bis-amidine (BAM) organocatalysts can furnish the building blocks for the total synthesis of the tridecapeptide, antiviral natural product feglymycin.

58. Development of small molecule modulators of Kv3.1 channels to restore PV interneuron dysfunction in neurodevelopmental and epileptic disorders

Rahul Chandrappa, Vaishali Satpute, Emily Days, Liangping Li, Corbin Whitwell, Paul Spearing, Kwangho Kim, Brad A. Grueter, Craig W. Lindsley, Alex Waterson, and Jerod S. Denton

Developmental and epileptic encephalopathies (DEEs), autism spectrum disorder (ASD), and schizophrenia are neurodevelopmental conditions that significantly impair quality of life and impose a substantial socioeconomic burden. Genetic mutations and environmental insults can lead to these conditions, and a common pathophysiological feature across these disorders is an abnormal excitatory-to-inhibitory (E/I) synaptic transmission ratio, suggesting that pharmacological strategies aimed at restoring E/I balance may offer therapeutic benefit. GABAergic parvalbumin-expressing fast-spiking interneurons (PV-INs), which play a pivotal role in regulating inhibitory tone and coordinating high-frequency oscillations essential for cognition, sensory processing, and motor control, are frequently disrupted in these disorders. The voltage-gated potassium channel Kv3.1b, predominantly expressed in PV-INs, enables their high-frequency firing and represents a promising, cell-specific drug target. While previous efforts have focused on developing Kv3.1b potentiators to enhance PV-IN excitability in KCNC1 loss-of-function (LoF) associated diseases, recent genetic and clinical findings have highlighted the added need for Kv3.1b inhibitors in the context of KCNC1 gain-of-function (GoF) mutations, which lead to hyperactive Kv3.1b channels and impaired neuronal function. Thus, our drug discovery program has expanded to include both Kv3.1b potentiators and inhibitors, tailored to the underlying channel dysfunction in distinct patient populations. We employed a molecular target-based approach to identify novel small molecules that modulate Kv3.1b activity. Using a fluorescence-based high-throughput screening (HTS) assay, we screened over 50,000 compounds from the Vanderbilt Institute of Chemical Biology (VICB) library, identifying potential potentiator and inhibitor hits. Additionally, for Kv3.1b activators, we plan to synthesize and evaluate analogs of known Kv3.1 modulators (e.g., RE1/AUT1), aiming to improve potency, selectivity, and pharmacokinetic properties, but none of them have surpassed the potency of the parent compound yet. For inhibitors, due to the lack of potent and selective Kv3.1b inhibitors in the field, we aim to develop analogs of effective inhibitors identified in our screen. Next steps include automated patch-clamp electrophysiology to validate both potentiator and inhibitor hits, followed by ex vivo brain slice recordings to assess their ability to restore PV-IN excitability and normalize E/I balance in disease-relevant circuits, including the prefrontal cortex and nucleus accumbens. An iterative medicinal chemistry campaign will further optimize lead compounds for in vivo efficacy and drug-like properties. This dual-pronged strategy—developing both Kv3.1b potentiators and inhibitors—offers a precision medicine approach to modulating PV-IN function across a spectrum of neurodevelopmental disorders.

59. Development of NAPE-PLD activators for the treatment of cardiometabolic diseases

Reza Fadaei, Azuah Gonzalez*, Kwangho Kim*, Liangping Li*, Ben Levine, Plamen P. Christov, Somnath Jana, Emily Days, Claire Hanson, David Baughman, Margaret A. Read, Joshua A. Bauer, Gary Sulikowski, Alex Waterson, Amanda C. Doran, Sean S. Davies

N-acyl-phosphatidylethanolamine hydrolyzing phospholipase D (NAPE-PLD) catalyzes the final step in the formation of N-acyl-ethanolamines (NAEs), a family of bioactive lipids that include palmitoylethanolamide (PEA), oleoylethanolamide (OEA), and anandamide (AEA). By a closely related mechanism, NAPE-PLD also catalyzes the inactivation of N-aldehyde modified phosphatidylethanolamines (NALPEs), a family of proinflammatory lipids formed during lipid peroxidation. Treating mice fed a Western Diet (high-fat, high cholesterol) with PEA or OEA inhibits excess food intake, weight and fat gain, hepatosteatosis, and progression of atherosclerosis. A key driver of atherosclerosis is impaired efferocytosis, the process by which macrophages and other phagocytic cells clear apoptotic cells from sites of injury and wounding. Impaired efferocytosis leads to the development of large necrotic cores within lesions, creating atherosclerotic plaques vulnerable to rupture. In human coronary arteries, vulnerable plaques have reduced NAPE-PLD expression compared to healthy arteries, suggesting that reductions in NAPE-PLD expression contribute to impaired efferocytosis. We found that bone marrow derived macrophages (BMDM) where NAPE-PLD is genetically deleted or chemically inhibited have reduced efferocytosis capacity compared to wild-type or vehicle treated BMDM when measured in a cultured cell assay of efferocytosis. PEA and OEA enhance efferocytosis, while Isolevuglandin-phosphatidylethanolamine (IsoLG-PE), one of the major species of NALPEs, inhibits efferocytosis. RNA-Seq studies revealed that overnight treatment of BMDM with the NAPE-PLD inhibitor LEI-401 markedly increases expression of inflammatory genes (Adgre1, Cd14, Ccr-1,-3,-5; Ccl-2, -5,-7, -8, -9, -12; Cxcl-2, -3, 2, Saa3, Mmp-9,-13) and oxidized LDL scavenger receptors (Msr1, Cd36, and Marco). Small molecule activators of NAPE-PLD are a potential strategy to restore macrophage efferocytosis capacity and thereby treat or prevent atherosclerosis. High-throughput screening of 40K compounds for effects on NAPE-PLD activity identified several benzothiazole phenylsulfonyl-piperidine carboxamides including VU'534 that increased NAPE-PLD activity. Treating BMDM with VU'534 increased their efferocytosis capacity in the cultured cell efferocytosis assay. Unfortunately, VU'534 has relatively poor cell penetrance and is rapidly metabolized in vitro by microsomes, making it a poor candidate for use in vivo. To identify activators with greater potency and efficacy, and with more favorable in vitro DMPK characteristics, we undertook a medicinal chemistry campaign to examine the effects of modifying the benzothiazole moiety, the piperidine linker, and the sulfonylamide tail of VU'534. We have identified compounds which show greater potency and efficacy than VU'534 in both a cellular NAPE-PLD activity assay and the cultured cell efferocytosis assay. Future studies in mice will measure the in vivo DMPK characteristics of these new compounds and their ability to enhance efferocytosis in vivo and reduce atherosclerosis.

60. Bioinformatic identification & characterization of graspetide tailoring enzymes

Riley Carter

Graspetides are a class of ribosomally synthesized and post-translationally modified peptide (RiPP) natural products that contain macrolactone and/or macrolactam linkages installed between amino acid side chains in a peptide. RiPP biosynthesis involves a genetically encoded precursor peptide first being translated by the ribosome followed by the installation of various modifications using tailoring enzymes. These linkages are installed by a group of ATP-grasp ligases termed graspetide synthetases. Graspetide synthetases often install multiple linkages within a single precursor peptide, producing multi-macrocyclic structures with either interlocked rings or rings within rings. The resulting complex structure are often protease resistant, a crucial feature in peptide-based therapeutics. Additional modifications have been shown to modulate the biological activity of certain graspetides. While other natural products can often contain additional biosynthetic tailoring and modifications, the only additional modifications that have been observed on graspetides are methylations and acetylations. As such, genome mining was utilized to prioritize the characterization of graspetide biosynthetic gene clusters that contain additional biosynthetic enzymes. In addition to determining the structure and linkage pattern of each graspetide, investigations were conducted into the requirements and timing of the additional biosynthetic proteins.

61. Cytochrome bd inhibitors show efficacy in altering the growth of Uropathogenic Escherichia coli

Samantha Kasbohm, Sarah Comer, Zachary R. Austin, Ian Romaine, Gary Sulikowski, Maria Hadjifrangiskou

Annually, the number of urinary tract infections (UTIs) is around 150 million3, and the primary mode of treatment for these is with antibiotics. However, as the number of antibiotic-resistant bacteria increases, the need for novel therapeutics to treat them increases as well. A potential solution is targeting bacterial respiration using small-molecule inhibitors. One target of interest is cytochrome bd, a terminal oxidase found in Uropathogenic Escherichia coli (UPEC), that has been shown to promote UPEC survival in the microaerobic conditions found during infection1. To test whether cytochrome bd is a viable therapeutic target, a suite of cytochrome bd inhibitors were generated by the VICB Molecular Design and Synthesis Center. These compounds were based on previously known cytochrome bd inhibitors, including CK-2-63, which has been shown to alter the growth of Mycobacterium tuberculosis2. To analyze the efficacy of these compounds, UTI89, a common lab strain of UPEC, was treated with 100 ?M of each compound and grown for 12 hours, along with untreated UTI89. From the initial screening of all compounds, Compounds MDSC-649-08 and MDSC-649-09 created the largest decrease in maximal OD600 compared to the rest of the potential cytochrome bd inhibitors. Compound 09 also showed a dose dependent effect on UTI89 when tested at different concentrations. These findings show that Compound MDSC-649-09 is a promising candidate for further study, and next steps include determining whether the compound is interacting directly with cytochrome bd by purifying the complex and observing its binding profile as well as testing it in combination with marketed antibiotics.

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62. Characterization and pharmacological probing of lysosomal cation channel TMEM175.

Samantha Le, KJ Li, Vaishali Satpute, Emily Days, Jerod S. Denton

TMEM175 is a lysosomal-resident proton (H+) and potassium (K+) ion channel that is critical for regulating lysosomal membrane potential and pH homeostasis. These functions are essential for maintaining the acidic environment required for organelle function and integrity. Given growing evidence linking TMEM175 loss-of-function (LOF) to neurodegenerative diseases such as Parkinson's disease, there is a pressing need to develop small-molecule activators for exploring TMEM175's therapeutic potential. To this end, we screened a library of 960 FDA-approved drugs for TMEM175 modulators using a HEK-293 cell line overexpressing TMEM175 at the plasma membrane and fluorescence-based thallium (Tl+) flux assay. The screen identified several novel activators, including the cysteinyl leukotriene 1 (CysLT1) receptor antagonists, pranlukast and montelukast. Dose-response experiments of pranlukast, montelukast, zafirlukast, and the known TMEM175 activator, DCPIB, revealed a rank-order potency and efficacy of DCPIB ~ zafirlukast > montelukast ~ pranlukast. Kinetic analysis revealed that DCPIB and zafirlukast cause immediate TMEM175 activation whereas montelukast- and pranlukast-dependent activation is much slower. Inhibition of AKT with MK2206, which activates TMEM175 through protein-protein interactions, has no effect on activation. In silico docking identified a conserved ligand interaction site at the inter-subunit interface of TMEM175. Mutagenesis analysis of this cleft domain abolished drug sensitivity and resulted in constitutive channel activation, indicating this region is critical TMEM175 gating. Our study identifies zafirlukast as a novel TMEM175 activator whose potency is similar to that of DCPIB and raises important questions about TMEM175 channel gating.

63. Development of Photometabolomics for the Discovery of Light Sensitive Natural Products

Seth R Patterson, Henry A.M. Schares, Brian O. Bachmann

Natural product discovery is a pivotal area of research aimed at identifying biologically active compounds from natural sources, such as plants, marine organisms, and microorganisms. These compounds often exhibit diverse pharmacological properties and have the potential to serve as lead compounds in drug discovery and development. Precedent within the lab has shown that polyene macrolactam ciromicin A will isomerize to ciromicin B upon exposure to visible light, with the two isomers displaying different bioactivity. This observation gave rise to the use of photochemical reactivity as an initial means for discovery of polyene macrolactams, coined photometabolomics. Photometabolomics consists of using LC-MS and multivariable statistical analysis to compare dark and light samples of the crude metabolomes of proposed polyene macrolactam producing bacterial strains. Work is currently underway to optimize conditions for exposure to light including amount of time exposed, distance from light source, and ambient temperature of the sample environment. Doxorubicin conjugated to a photocleavable biotin is spiked into samples and used as a control to ensure sufficient light exposure. The goal of the project is to develop a workflow that allows the discovery of photoreactive natural products within a crude extract and determine the differential bioactivity of the photoisomers.

64. Exploring substrate tolerance of a graspetide synthetase

Sharon Roth

Macrocyclic peptides are attractive modalities for drug discovery due to their ability to inhibit challenging targets, such as protein-protein interactions. Graspetides, a subclass of ribosomally synthesized and post-translationally modified peptides (RiPPs), feature multiple sidechain-to-sidechain macrocycles installed by ATP-grasp ligases. These enzymes catalyze the formation of both ester and amide bonds, and different ATP-grasp enzymes install distinct crosslinking patterns. In this work, we report the discovery of a new graspetide natural product from Thermoanaerobacterium thermosaccharolyticum. Using heterologous expression in E. coli, we identified a peptide, TheA, bearing five crosslinks installed by the ATP-grasp ligase TheB. To evaluate TheB as a tool for generating diverse macrocyclic structures, we first reconstituted its activity in vitro. We then assessed the specificity of TheB by conducting an alanine scan of the TheA core peptide. TheB was able to install at least one crosslink on each single-alanine variant, indicating positional tolerance at each core residue. However, scrambled versions of the core sequence—containing the same amino acids in different orders—were not modified by TheB, suggesting requirements for sequence order or conformational features. These results demonstrate that TheB exhibits context-dependent substrate promiscuity. Further substrate mutagenesis and structural modeling will provide insight into the factors determining substrate

recognition and modification. Understanding these rules will enable the engineering of novel graspetides as customizable macrocyclic scaffolds for therapeutic applications.

65. Mining underutilized resources for hidden microbial metabolism
Shravan Dommaraju

Microbial biosynthesis provides tremendous medicinal and biotechnological value. Access to genomic information for rare and understudied microbes has exploded, so tools for functional annotation and prediction are required to extract useful information. A staggering amount of public genomic data is deposited in short contigs and lacks annotation. We developed an advanced genome mining tool, MetaRODEO, to efficiently mine these unannotated and "low-quality" (meta)genomic data as a putative untapped biosynthetic reserve. We validated MetaRODEO by characterizing metabolites encoded by uncultured human-associated and coral-reef-associated microbes. Knowing that these “low-quality” data could lead to bona fide new metabolites, we targeted biosynthetic pathways from phylum Acidobacteria, a highly prevalent and biosynthetically rich taxon, which is difficult to culture. Through our efforts, we characterized biosynthesis of an Acidobacterial lasso peptide and multiple lipopeptides, representing some of the first unique natural products from this phylum. We further targeted extremophiles, which, owing to low culturability and habitat access, are inherently difficult to study. We first characterized a pathway from a hydrothermal vent metagenome, which produces a hypermodified, hyperanionic peptide bearing 14 phosphorylations. In addition, we examined a pathway from a hypersaline lake environment, which produces a peptide bearing up to 17 GABAylations. Biosynthesis of this “gabatide” repurposes eukaryotic tubulin-modifying enzymes to install a novel modification onto an unnatural prokaryotic substrate. These hyperanionic, hypermodified peptides are widespread throughout ecosystems and microbial taxa, suggesting they may play important evolutionary roles. Altogether, our efforts with MetaRODEO profiled an untapped source of public genomic data, resulting in a large dataset of novel biosynthetic pathways, the production of >10 new metabolites, and discovery of unprecedented biosynthetic enzymes.

66. Magnetic Bead Electrochemical Biosensor Assesses the Influence of Human Milk Oligosaccharides on Interleukin-1β Inflammation in THP-1 Macrophages for Reproductive Health
Victoria A. Federico, Hannah A. Richards, Julie A. Talbert, Jocelyn R. Leal, David E. Cliffel, Steven D. Townsend

Human milk oligosaccharides (HMOs) are structurally diverse carbohydrates known to have antimicrobial and antibiofilm properties against pathogens such as Group B Streptococcus (GBS), a major cause of perinatal infections and preterm birth. Recent studies suggest that HMOs may also modulate immune responses by inhibiting inflammatory cytokines like interleukin-1β (IL-1β). This study aims to evaluate the impact of HMOs on IL-1β secretion in THP-1 macrophages and to compare the effectiveness of a Magnetic Bead Electrochemical Sandwich Assay (MBESA) with traditional enzyme-linked immunosorbent assay (ELISA) techniques. The MBESA can quantify IL-1β levels following HMO treatment, enabling the assessment of both the immunomodulatory effects of HMOs and the analytical performance of this electrochemical biosensing method. These findings will provide a foundational step toward exploring HMOs as therapeutic agents against inflammatory-related diseases, while also highlighting the potential of the MBESA as a rapid and sensitive alternative to conventional immunoassays. Future work will integrate this IL-1β electrochemical biosensor with a fetal membrane on-a-chip model to investigate the therapeutic potential of HMOs for reproductive health applications.

67. Accessing A Unique Class of Antibiotics: Total Synthesis of the Aminoglycoside Plazomicin
Valentina Guidi, Aaron Xu, Daria E. Kim

Aminoglycosides are a class of potent, broad-spectrum antibiotic molecules used clinically for the treatment of severe bacterial infections. Plazomicin, a therapeutic agent against urinary tract infections, is of particular interest because unlike other aminoglycosides, it is resistant to most aminoglycoside-modifying enzymes, thus lowering its susceptibility to bacterial resistance. Plazomicin’s structure consists of an unsaturated aminomonosaccharide residue, C4-methylated aminoarabinopyranoside residue, and 2-deoxystreptamine core. Traditionally, the synthesis of plazomicin has relied on the derivatization of the less potent natural isolate sisomicin, which highlights a major limitation in aminoglycoside synthesis: the difficulty in directly accessing structural modifications which instigate antibiotic resistance. We aim to circumvent this limitation by developing a total synthesis of plazomicin, allowing for a concise construction of the molecule via tailored modifications. We will target plazomicin’s three core fragments separately and link the fragments via two glycosylation events. The 2-deoxystreptamine core will be synthesized from homoserine and involve a stereoselective fragment coupling and ring closing via a Henry reaction. The synthesis of the arabinose derivative will involve the key regioselective radical methylation of the C4 carbinol. Lastly, the unsaturated aminomonosaccharide residue will be accessed through a glutamic-acid-derived gamma-lactone and feature a C4-C5 olefin oxidation and cross-coupling to install the ethanolamine motif. Because most aminoglycosides are comprised of similar building blocks, the key steps of this plazomicin synthesis could be later utilized to access a variety of aminoglycosides.

68. Activation of metabotropic glutamate receptor 7 rescues NMDAR antagonist-induced cognitive deficits: implications for psychotic disorders
Niki Harris, Mac Meadows, Arisa Timoll, Shalini Dogra, Colleen Niswender

Schizophrenia is a neuropsychiatric disorder characterized by positive, negative, and cognitive symptoms, affecting roughly 1% of the global population. Current antipsychotic medications, which primarily target dopaminergic and serotonergic systems, are effective against positive symptoms; however, these medications display limited efficacy against negative and cognitive symptom domains. Therefore, while positive symptoms may be attributed to dopaminergic hyperfunction, growing evidence instead suggests that glutamatergic dysfunction may drive cognitive and negative symptoms. In support, models of glutamatergic N-methyl-D-aspartate receptor (NMDAR) hypofunction via administration of NMDAR antagonists (i.e. PCP, MK-801) in both healthy human and animal subjects induces cognitive and behavioral deficits similar to those observed in patients with schizophrenia. Interestingly, various clinical studies demonstrate that schizophrenia and other neurodevelopmental disorders characterized by cognitive deficits are associated loss-of-function variants in GRM7 – the gene encoding for mGlu7. Thus, group III metabotropic glutamate (mGlu) receptors, including receptor subtype 7 (mGlu7), have emerged as promising targets specifically for these cognitive and negative symptoms due to their potential to restore abnormal glutamatergic signaling. Based on these observations, we hypothesize that LSP2-9166, an mGlu4/7 agonist, will correct deficits in cognitive and social behaviors in a mouse model of NMDAR hypofunction. Preliminary data suggest that administration of LSP2-9166 (10 mg/kg; i.p.) corrects spatial working memory deficits and hyperlocomotive effects induced by MK-801 (0.18 mg/kg, i.p.). Based on dose required for efficacy, these data indicate that LSP2-9166 exerts these positive effects on cognition primarily through activation of mGlu7. Ultimately, we anticipate that these studies will establish a potential therapeutic role for mGlu7 modulation in correcting cognitive and behavioral deficits in a model of glutamatergic dysfunction, positioning this strategy as a viable approach to target schizophrenia-like pathophysiology.

69. Assessing Enzymatic Activity of Adenosine Deaminase 2 (ADA2) and Its Variants via Nano Luc-Integrated Indophenol Blue Assay
Yijin Shen, Lea A. Barny, Minsoo Kim, Lars Plate

Adenosine deaminase 2 deficiency (DADA2) is an autosomal recessive autoinflammatory disease caused by loss-of-function mutations in the ADA2 protein, a secreted enzyme that converts adenosine to inosine and releases ammonia as a byproduct. Clinical manifestations include systemic inflammation, vasculitis, immunodeficiency, and bone marrow failure. Here, we study a non-patient-derived gain-of-function variant, K400D, associated with increased activity and stability in mice, making it a promising candidate for enzyme replacement therapy. To thoroughly

characterize the catalytic properties of this mutant compared to others, we developed a semi-automated workflow using a liquid handler to measure secretion and activity. We aimed to (1) confirm whether K400D exhibits increased catalytic activity relative to wild-type and other mutants, (2) determine whether this increase results from higher intrinsic activity or secretion levels, and (3) assess the functional importance of residue 400 through substitution mutants. We engineered NanoLuc-tagged ADA2 constructs and transiently transfected them into HEK293T cells. Secretion levels were measured via luminescence, and catalytic activity was assessed using an indophenol-based colorimetric assay. Normalization of ADA2 protein levels enabled comparison of catalytic activity per unit of enzyme secreted. Wild type ADA2 exhibited ~1.8-fold higher abundance than K400D in both media and lysate, suggesting greater overall expression or stability. K400D demonstrated ~45.2% higher extracellular activity compared to wild type, suggesting a potential gain in catalytic efficiency or extracellular stability. Our preliminary findings suggest that K400D enhances extracellular ADA2 activity independently of total secretion, indicating a potential gain in catalytic efficiency. Together, these results highlight the value of quantitative activity profiling in guiding ADA2 variant characterization and therapeutic enzyme development for DADA2.

70. Time-Resolved Interactome Profiling to Track Time-Dependent Interac9ons of Proteostasis Factors Responsible for Membrane Protein Folding and Trafficking

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While protein structure predic9on has greatly improved in recent years due to AI advancements, our understanding of how proteins fold is s9ll incomplete. The cell employs the proteostasis network (PN), made up of hundreds of chaperones, cochaperones, and folding factors, which assist with folding and trafficking properly folded proteins, while degrading misfolded proteins. Amino acid mutations can result in protein misfolding, imbalances in PN surveillance, and aggregation which is associated with numerous diseases. Although many key chaperones and proteastasis dependencies are known for client proteins, the exact temporal sequence of cellular protein folding is difficult to trace with current methods. To better understand the time dependent nature of folding, Time-Resolved Interactome Profiling (TRIP) was developed in the Plate lab to track the folding and secretion of the thyroid pro-hormone thyroglobulin. So far, TRIP has only been employed for secreted proteins, hence my focus has been on making the TRIP methodology amenable to membrane proteins. The ATP-binding cassette sub-family D member 1 (ABCD1), which transports very long chain fatty acids (VLCFA) from the cytosol into the peroxisomal lumen, was chosen as a model membrane protein. The original TRIP method has been modified by switching from noncanonical amino acid (ncAA) incorporation of L-Homopropargylglycine (Hpg) coupled with Cu-Catalyzed Azide–Alkyne Cycloaddi9on (CuAAC) to ncAA incorporation of L-Azidohomoalanine (Aha) coupled with Strain-Promoted Azide-Alkyne-Cycloaddition (SPAAC), which is copper free. Further optimization has focused on reducing labeling time for increased 9me resolu9on, reducing click reaction background by blocking free cysteines with iodoacetamide (IAA), and increasing magnetic nanobody conjugated beads, that recognize a GFP tag on ABCD1, for beLer pulldown efficiency. Additionally, crosslinking of protein-protein interac9ons was confirmed via liquid chromatography-tandem mass spectrometry (LC-MS/MS). Using wild-type ABCD1, the method was validated by 9me-course data which revealed time-dependent interactions of key proteostasis factors.

71. LassoPred: a tool to predict the 3D structure of lasso peptides

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Lasso peptides (LaPs), characterized by their entangled slipknot-like structures, are a large class of ribosomally synthesized and post-translationally modified peptides (RiPPs), with examples functioning as antibiotics, enzyme inhibitors, and molecular switches. Despite thousands of LaP sequences predicted by bioinformatics, only around 50 distinct LaPs have been structurally characterized in the past 30 years. Existing computational tools, such as AlphaFold2, AlphaFold3 and ESMfold, fail to accurately predict LaP structures due to their irregular scaffold featuring a lariat knot-like fold and the presence of an isopeptide bond. To address this challenge, we developed LassoPred, designed with a classifier to annotate the ring, loop, and tail of an LaP sequence and a constructor to build a 3D structure. Leveraging LassoPred, we predict the 3D structures for 4749 unique LaP core sequences, creating the largest in silico-predicted lasso peptide structure database to date. LassoPred is publicly available through a web interface (https://lassopred.accre.vanderbilt.edu/) and a command-line tool, supporting future structure-function relationship studies and aiding in the discovery of functional lasso peptides for chemical and biomedical applications.

72. Sulfur incorporates into E. coli ribosome by a proteinaceous sulfur donor

Yanqing Xue, Douglas A. Mitchell

Thioamides are commonly used peptide bond isosteres in medicinal chemistry, yet naturally occurring thioamides remain underexplored. One of the few known examples is the methyl-coenzyme M reductase (MCR) from methanogens, which harbors a thioamide in one of its subunits. Recently, high-resolution cryo-electron microscopy of the Escherichia coli 70S ribosome unambiguously revealed a thioamide modification on ribosomal protein uL16, whose thioamide-containing loop projects into the peptidyl transferase center.

In MCR, thioamidation proceeds via backbone amide O-phosphorylation catalyzed by a YcaO enzyme, followed by nucleophilic attack by a bisulfide (HS^-) species, which is hydrolyzed from a thiocarboxylated ThiS (ThiS-COSH) by a TfuA partner protein. Our previous work characterized thioamidation of uL16 catalyzed by an E. coli YcaO using exogenous Na_2S as a sulfur donor (Andrew Rice, Yanqing Xue et al., unpublished). However, given the toxicity of free HS^- to a range of cellular enzymatic processes, and the high abundance of ribosome that would necessitate substantial HS^- concentrations, it is unlikely that free HS^- serves as the physiological sulfur source.

Consistently, deletion of the mstA gene, which accounts for over 90% of endogenous HS^- production in E. coli, has little impact on ribosome thioamidation. Furthermore, E. coli lacks a TfuA homolog, and deletion of thiS does not impair thioamide formation. These findings suggest a mechanistic divergence in sulfur incorporation between uL16 and MCR.

Here, we characterize the involvement of the cysteine desulfurase IscS, which is an essential component of the iron-sulfur cluster biosynthetic machinery, in the sulfur incorporation process, and implicate the IscS persulfide (IscS-SSH) as the direct sulfur donor for uL16 thioamidation. Reduction of sulfane sulfur (S^0) in IscS-SSH to S^{2-} in thioamide likely requires ferredoxin as an electron donor, evidenced by impaired thioamidation in Δfdx mutant. These results elucidate the sulfur incorporation pathway for ribosomal thioamide formation and increase our appreciation of persulfide chemistry in vivo.

73. MECHANISTIC INSIGHTS INTO AN INTERSTRAND DNA CROSSLINK GLYCOSYLASE THAT AIDS ACINETOBACTER BAUMANNII PATHOGENESIS

Yujuan Cai, Dillon E. Kunkle, Eric P. Skaar, Brandt F. Eichman

DNA glycosylases initiate the base excision repair (BER) pathway by excising aberrant nucleobases from the phosphoribose backbone. Previously, we characterized E. coli YcaQ as an DNA glycosylase that unhooks guanine N7-linked ICLs as an alternative to NER (Bradley et al, 2020, Nucleic Acids Res 48: 7005). Similarly, we showed that the YcaQ ortholog in Acinetobacter baumannii, which we have named AlkX, excises ICLs derived from the nitrogen mustard mechlorethamine and protects the bacteria from alkylation and acid stress (Kunkle, Cai et al, 2024, PNAS 121: e2402422121). A. baumannii is a nosocomial pathogenic bacteria and is the fifth leading cause of antimicrobial resistance-associated deaths globally. alkX is maintained in A. baumannii clinical isolates but is not encoded in at least one nonpathogenic Acinetobacter strain. We showed in vivo that AlkX loss sensitizes cells to DNA crosslinking agents and impairs A. baumannii colonization of the lungs and dissemination to distal tissues during pneumonia, suggesting that AlkX participates in A. baumannii pathogenesis and protects the bacterium from stress conditions encountered in vivo. Despite the importance of the YcaQ/AlkX family of glycosylases in bacteria, the mechanisms by which this unique enzyme recognizes and unhooks ICLs are unknown. Here, we report the crystal

structure of Thermobifida fusca (Tfu) AlkX in complex with DNA containing an abasic site analog, mimicking the product of the DNA glycosylase reaction. The structure reveals a loop from the β-barrel domain that inserts into the minor groove and places a conserved tyrosine in proximity of the abasic nucleotide. In vitro and in vivo assays showed the deletion of this DNA binding loop abrogated the base excision activity and the robustness of the strain under DNA damage stress. These results are an important initial step in determining the mechanism by which BER affects A. baumannii pathogenesis and multi-drug resistance.

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