2023
August 8th

VANDERBILT INSTITUTE OF CHEMICAL BIOLOGY
STUDENT RESEARCH SYMPOSIUM
VANDERBILT STUDENT LIFE CENTER

MORNING SESSION	
8:30 am - 8:35 am	**Welcome Remarks: Gary Sulikowski (Director, Vanderbilt Institute for Chemical Biology)**
Oral Session I *Therapeutics, Translation and Chemical Synthesis* (Jennifer Wurm, Chair)	
8:35 am – 8:55 am	**Sabrina Spicer (Townsend Lab)** *Pour some sugar on me: underscoring the antimicrobial applications of human milk oligosaccharides*
8:55 am – 9:00 am	**Break**
9:00 am – 9:20 am	**Rachana Tomar, Ph.D. (Stone Lab)** *Structural basis of error-prone replication opposite urea lesion by human DNA polymerase η*
9:20 am – 9:25 am	**Break**
9:25 am – 9:50 am	**Richard Armstrong Prize for Research Excellence.** **Eli McDonald, Prize Runner Up (Plate Lab)** *Examining PFKL Dynamics with Site Specific Unnatural Amino Acid Incorporation*
9:50 am -10:00 am	**Break**
Oral Session II *Molecular Discovery and Systems Analysis* (Crissey Cameron, Chair)	
10:00am-10:20 am	**Brandon Baer, Ph.D. (Bastarache Lab)** *Overexpression of alveolar epithelial tissue factor promotes maintenance of lung barrier integrity in ALI*
10:20am-10:25 am	**Break**
10:25am- 10:45 am	**Kyle Enriquez (Skaar Lab)** *Temporal modelling of the biofilm lifecycle (TMBL) establishes kinetic analysis of plate-based bacterial biofilm dynamics*
10:45am- 10:50am	**Break**
10:50am-11:15 am	**Richard Caprioli, Ph.D. (Professor, Biochemistry; Director, Mass Spectrometry Research Center)** *Next Generation Imaging Mass Spectrometry: Molecular Microscopy for Biology and Medicine*
11:15am-11:30 am	**Break**
11:30am-11:40am	VICB Citation Winner Flash Talk: **Seth Zost, Ph.D. (Carnahan and Crowe Lab)-** *Potentially neutralizing and protective human antibodies against SARS-CoV-2*
11:40am – 12:10pm	**Matthew Nelson, Ph.D. (Vice President, Genetics and Genomics, Deerfield Discovery and Development)** *Expanding insights into the value of genetic evidence for drug discovery and the myth of peak genetics*
LUNCH BREAK 12:05 pm – 1:00 pm	
12:30 pm – 1:00 pm	**Q & A with Dr. Yates (RSVP-only event)**
AFTERNOON SESSION	
Oral Session III *Richard Armstrong Prize for Research Excellence* (Ruben Torres, Chair)	
1:00 pm - 1:30 pm	**Lars Plate, Ph.D. (Assistant Professor, Chemistry and Biological Sciences)** *Spatial-temporal control of secretory proteostasis*
1:30 pm - 2:00 pm	**Rachael Wolters, Prize Winner (Crowe Lab)** *Title TBD*
2:00 pm - 2:10 pm	**Break**

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Oral Session IV *Keynote* (Laurel Bellocchio, Chair)	
2:10 pm - 3:10 pm	**John Yates III, Ph.D. (Professor of Molecular Medicine and Neuroscience at The Scripps Research Institute)** *Probing the in vivo structure of mutant CFTR*
3:10 pm – 3:15 pm	**Break**
3:15 pm – 4:00 pm	**"A.I. and Big Data- you can't ignore it!" Panel**
RECEPTION	
4:00 pm – 5:00 pm	**Poster session**

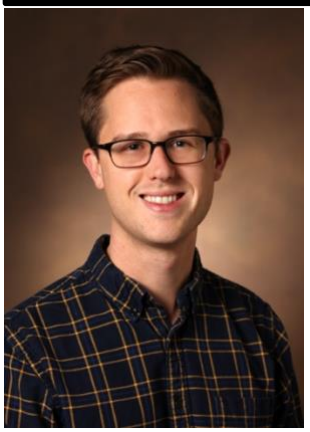
"Probing the *in vivo* structure of mutant CFTR "

John Yates III, Ph.D.
Class of 1942 Chair Professor
Departments of Chemistry and Molecular and Cell Biology
Director, Chemical Biology Graduate Program
University of California, Berkeley

John R. Yates is the Ernest W. Hahn Professor in the Departments of Molecular Medicine and Neurobiology at The Scripps Research Institute. He received a B.A in Zoology and an M.S. in Chemistry from the University of Maine at Orono. He obtained his Ph.D. in Chemistry at the University of Virginia in the laboratory of Donald F. Hunt with a dissertation entitled *Protein Sequencing by Tandem Mass Spectrometry*. He performed postdoctoral research in the laboratory of Leroy E. Hood at California Institute of Technology. At the University of Washington, he obtained the rank of Associate Professor with tenure before moving to The Scripps Research Institute. His research interests include development of integrated methods for tandem mass spectrometry analysis of protein mixtures, bioinformatics using mass spectrometry data, and biological studies involving proteomics. He is the lead inventor of the SEQUEST software for correlating tandem mass spectrometry data to sequences in the database and developer of the shotgun proteomics technique for the analysis of protein mixtures. His laboratory has developed the use of proteomic techniques to analyze protein complexes, posttranslational modifications, organelles and quantitative analysis of protein expression for the discovery of new biology. Many proteomic approaches developed by Yates have become a national and international resource to many investigators in the scientific community.

He has received the American Society for Mass Spectrometry research award, the Pehr Edman Award in Protein Chemistry, the American Society for Mass Spectrometry Biemann Medal, the HUPO Distinguished Achievement Award in Proteomics, Herbert Sober Award from the ASBMB, and the Christian Anfinsen Award from The Protein Society, the 2015 ACS's Analytical Chemistry award, 2015 The Ralph N. Adams Award in Bioanalytical Chemistry, the 2018 Thomson Medal from the International Mass Spectrometry Society, the 2019 John B. Fenn Distinguished Contribution to Mass Spectrometry award from the ASMS, and the 2019 HUPO Award in Discovery. He was ranked by Citation Impact, Science Watch as one of the Top 100 Chemists for the decade, 2000-2010. He was #1 on a List of Most Influential in Analytical Chemistry compiled by *The Analytical Scientist* 10/30/2013 and is on the List Of Most Highly Influential Biomedical Researchers, 1996-2011, *European J. Clinical Investigation* 2013, 43, 1339-1365 and the Thomson Reuters 2015 and 2019 List of Highly Cited Scientists. He has published over **900** scientific articles with **>161,000** citations, and an H index of **197 (Google Scholar)**. Dr. Yates served as an Associate Editor at *Analytical Chemistry* for 15 years and is currently the Editor in Chief at the *Journal of Proteome Research*.

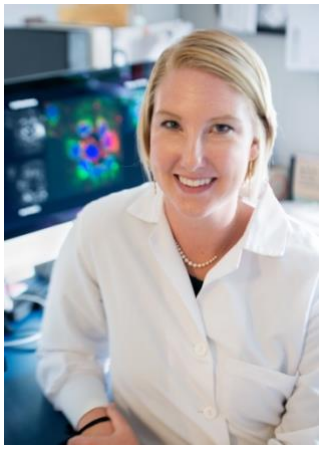
8th August 2023 | *VICB CITATION WINNERS*



Potentially neutralizing and protective human antibodies against SARS-CoV-2.

Seth Zost, Ph.D. (Carnahan and Crowe lab). Seth graduated from the University of Pennsylvania in 2013 with a B.A. in Biochemistry and History and a M.S. in Chemistry. Following graduation, Seth joined the lab of Dr. Scott Hensley at the University of Pennsylvania and studied human antibody responses to influenza A viruses and the viral evasion of pre-existing immunity. Seth defended his Ph.D. in 2018. In the lab, Seth is continuing to study antibody responses against respiratory viruses with special interests in defining conserved sites on viral proteins that are targeted by human antibodies and identifying how prior exposure shapes human antibody responses against viruses like influenza and SARS-CoV-2.

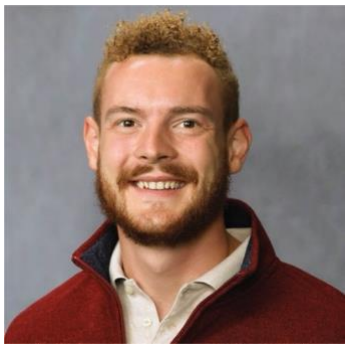
8th August 2023 | *RICHARD ARMSTRONG PRIZE FOR RESEARCH EXCELLENCE*



Rachael Wolters, James Crowe

Rachael Wolters is a graduate candidate in the department of Pathology, Microbiology, and Immunology at Vanderbilt, mentored by Dr. James E. Crowe, Jr. She obtained her B.S. in Animal Science from The University of Tennessee at Martin, where she conducted analytical chemistry research evaluating river water for fertilizer residue. Rachael went on to earn her Doctor of Veterinary Medicine from The University of Tennessee College of Veterinary Medicine, in Knoxville Tennessee.

During that time, she studied mechanisms of antimicrobial resistance (AMR) in *Staphylococcus pseudintermedius* and traveled to South Africa to the University of Pretoria to investigate epidemiologic patterns of AMR in dairy cattle. She was a member of the Cornell Veterinary Leadership Program in 2018 where she studied mechanisms of Zika virus replication. She is interested in emerging zoonotic infectious diseases, and animal models.



Examining PFKL Dynamics with Site Specific Unnatural Amino Acid Incorporation

Eli McDonald, Lars Plate

Eli grew up in Bettendorf, Iowa and completed his B.E. in Chemical Engineering at Vanderbilt University. His undergraduate research focused on the thermodynamics of phospholipid bilayer permeation and self-assembly at the Multiscale Modeling and Simulation Centre. Eli received the DAAD RISE Scholarship in 2016 to conduct summer research at the Laboratory of Engineering Thermodynamics where he studied the properties of salt solutions using molecular modeling. His current research project entails understanding the relationship between thermodynamic and kinetic regulation of CFTR folding and trafficking using Molecular Dynamics and chemical biology approaches. He is supported by the Vanderbilt Chemistry Biology Interface training grant on behalf of the Vanderbilt Institute of Chemical Biology.

Panel: A.I. and Big Data: You can't ignore it!

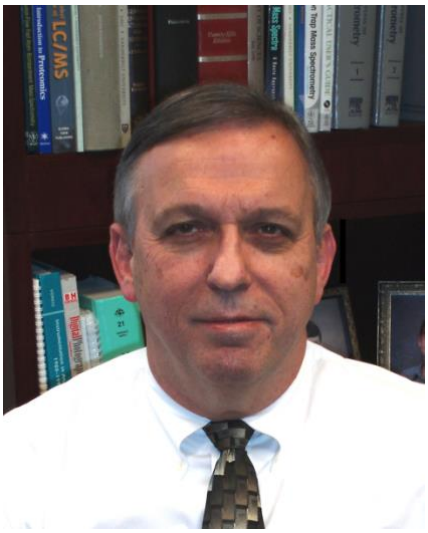
John Yates, PhD (Symposium Plenary Speaker, Scripps): John R. Yates is the Ernest W. Hahn Professor in the Departments of Molecular Medicine and Neurobiology at The Scripps Research Institute. He received a B.A in Zoology and an M.S. in Chemistry from the University of Maine at Orono. He obtained his Ph.D. in Chemistry at the University of Virginia in the laboratory of Donald F. Hunt with a dissertation entitled *Protein Sequencing by Tandem Mass Spectrometry*. His research interests include development of integrated methods for tandem mass spectrometry analysis of protein mixtures, bioinformatics using mass spectrometry data, and biological studies involving proteomics.

Matthew Nelson, PhD (Vice President, Genetics and Genomics, Deerfield Discovery): Matthew Nelson, Ph.D., is a Vice President, Genetics and Genomics, Deerfield Discovery and Development, and joined the firm in 2019. He is also Chief Executive Officer of Deerfield's affiliate, Genscience, a tech-focused company to improve integration of genetic evidence into drug discovery. Prior to joining Deerfield in 2019, Dr. Nelson spent almost 15 years at GlaxoSmithKline and was most recently the Head of Genetics. Prior to GlaxoSmithKline, Dr. Nelson was the Director of Biostatistics at Sequenom. He holds a Ph.D. in Human Genetics and an M.A. in Statistics from the University of Michigan and obtained his B.S. in Molecular Biology from Brigham Young University.

Hassane S. Mchaourab, PhD (Louise B. McGavock Chair, Department of Molecular Physiology and Biophysics, Vanderbilt University): Hassane S. Mchaourab was born in Beirut, Lebanon and attended the American University of Beirut where he majored in Physics and pursued a Master's degree that was interrupted by the civil war. He completed his Ph.D. in Biophysics at the medical College of Wisconsin under the supervision of James S. Hyde, focusing on the development of the theory and instrumentation of EPR spectroscopy. The theme of his research program since 1997 has been protein dynamics.

Allison S. Walker PhD (Assistant Professor, Department of Chemistry): Allison Walker received her Ph.D. in Chemistry from Yale University. Her lab uses machine learning and statistics to solve difficult problems in chemical biology. Her lab focuses on problems with existing large datasets or easily generated datasets, allowing them to take advantage of the wealth of genomic and metagenomic sequences. One area of focus is developing machine learning tools to facilitate engineering and discovery of natural products, which are excellent sources of bioactive molecules that can be used as therapeutics.

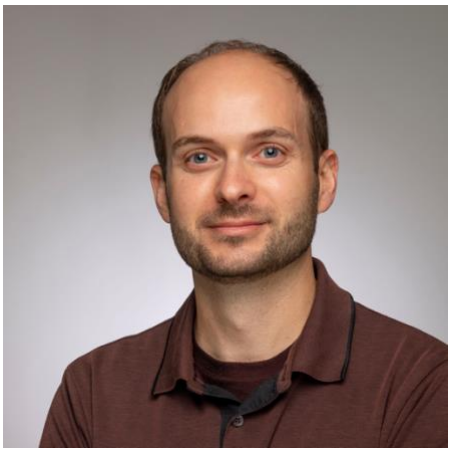
This panel will be moderated by Benjamin P. Brown, PhD (Research Assistant Professor, Department of Chemistry, Vanderbilt University): Benjamin P. Brown is a Research Assistant Professor in the Department of Chemistry. He completed his dissertation on the integration of ligand- and structure-based cheminformatics tools with protein dynamic modeling for drug design. He is interested in methods development research in computer-aided drug discovery (CADD), particularly as it relates to quantitative structure-activity relationship (QSAR) modeling, bidning free energy prediction, and selctivity modeling.



Next Generation Imaging Mass Spectrometry: Molecular Microscopy for Biology and Medicine

Richard Caprioli

Prof. Richard M. Caprioli is the Stanford Moore Chair in Biochemistry and Director of the Mass Spectrometry Research Center at Vanderbilt University School of Medicine. He is also Professor in the Departments of Chemistry, Medicine and Pharmacology. Dr. Caprioli moved to Vanderbilt in 1998 from the University of Texas. He has been President of the American Society for Mass Spectrometry and has won many awards in his field. He has pioneered the development of molecular microscopy using laser-based mass spectrometry technology and has applied it to biological and medical research projects.



Spatial-temporal control of secretory proteostasis

Lars Plate

A native of Germany, Lars Plate earned his BS at MIT working under JoAnne Stubbe. He completed his doctoral work at UC Berkeley in Molecular and Cell Biology in the group of Michael Marletta. He then performed postdoctoral work at the Scripps Research Institute in the labs of Jeff Kelly and Luke Wiseman. In 2017, he joined the Chemistry and Biological Sciences faculty at Vanderbilt University where his team studies the dynamics and coordination of protein interaction networks in diverse cellular processes. Research focuses on profiling the timing and coordination of protein interaction networks by combining chemical biology approaches with quantitative affinity purification-mass spectrometry methods. The group has applied these tools to understand the protein quality control defects that give rise to protein misfolding diseases, such as thyroglobulin-associated hypothyroidism and Cystic Fibrosis. Additionally, his team investigates how proteostasis processes play critical roles during flavivirus and coronaviruses infection and how these insights can be leveraged for development of host-targeted antivirals.

THERAPEUTICS AND TRANSLATION

Pour Some Sugar on Me: underscoring the antimicrobial applications of human milk oligosaccharides.

Sabrina Spicer, Jennifer A. Gaddy, and Steven D. Townsend

Human breast milk has long been coveted as the gold standard for infant nutrition. Containing a heterogenous mixture of fats, proteins, and other necessary nutrients, breast milk supplies complete nutrition to the neonate up to 2 years of life. Among the bioactive molecules found in human breast milk are human milk oligosaccharides (HMOs). While not digestible by the infant, these sugar molecules are widely known to promote the growth of commensal bacteria in the infant gut while suppressing the growth of pathogenic bacteria. We have previously characterized the impressive antimicrobial and antibiofilm activities of these molecules with gram positive pathogens at length. Interestingly, we have recently found that while the robust antibiofilm phenotype persists HMOs possess no antimicrobial power over *Acinetobacter baumanni*, an urgently threatening gram-negative pathogen. Biofilm-inhibiting compounds have recently gained attention as a potential chemotherapeutic strategy to prevent or dismantle *A. baumannii* biofilms and restore the utility of antimicrobial strategies. We have shown that human milk oligosaccharides (HMOs) have potent biofilm-inhibiting properties against a bank of *A. baumannii* clinical isolates. With these data in hand, we sought to test the utility of HMOs as antibiotic adjuvants against multidrug resistant isolates of *A. baumannii* to underscore the impact of biofilm formation in the antimicrobial resistance crisis. Our results indicate that the potent anti-biofilm activity of HMOs confounds antimicrobial resistance mechanisms to revive otherwise ineffective antibiotics, specifically those with cell surface targets including various carbapenems, a last line of defense antibiotic class for these types of infections. Investigations into molecular factors governing HMO-microbe and HMO-host-microbe interactions are of paramount interest. This includes assessment of proteomic differences upon HMO supplementation as well as the immunomodulatory properties of these molecules outside of the primitive infant gut. Preliminarily speaking, our data indicate that HMOs could be the light at the end of an exhausting tunnel of antimicrobial resistance.

CHEMICAL SYNTHESIS

Structural basis of error-prone replication opposite urea lesion by human DNA polymerase η

Rachana Tomar, Songlin Li, Martin Egli, and Michael P. Stone

Urea lesions are secondary damage products arising from thymine glycol (Tg) or 8-oxo-dG lesions in DNA. It equilibrates between a and b- deoxyribose anomers. They have potential to stall DNA replication both *in vitro* and *in vivo* and to induce point mutations. Their mutagenic potential has been proposed to arise due to their ability to form stable base pairing and stacking interactions with all four dNTPs during error-prone replicative bypass. Previously in vitro experiments reported human DNA polymerase η (hPol η) may incorporate all four dNTPs opposite urea and also extend DNA primer: template to full-length beyond urea lesion. Here, we provide X-ray crystallographic data for ternary replication bypass complexes of hpol h with incoming non-hydrolyzable dNTP analogs dAMPNPP, dCMPNPP, dGMPNPP and dTMPNPP opposite the urea lesion in a synthetic template: primer DNA, in the presence of Mg^{2+} ions. The structural data indicate that all four dNTP analogs may be accommodated in the flexible active-site pocket of hpol h. In each instance, the incoming dNTP analogs were oriented such that they were proximate to the 3'-hydroxyl of the primer terminus, confirming the potential for undergoing a successful nucleotidyl transfer reaction. The non-hydrolyzable dNTP analogs paired with the urea amino group and/or carbonyl oxygen through hydrogen bond formation between the Watson-Crick binding face of the respective dNTPs and the urea lesion. We further report through in vitro replication bypass assays that hPol η incorporate dATP with greatest efficiency and dTTP with lowest efficiency (dATP > dCTP > dGTP > dTTP). The structural data suggest that differential efficiencies for single dNTP incorporation by hpol h may be correlated with the stacking interactions, hydrogen-bonding interactions at active-site including mediated by Arg61, Gln38 residues and specific anomeric preference. Overall, these ternary complex structures represent error-free or error-prone replication of fragmented products arising from common oxidative DNA damage lesions.

MOLECULAR DISCOVERY

Overexpression of alveolar epithelial tissue factor promotes maintenance of lung barrier integrity in ALI

Brandon Baer, Nathan D. Putz, Noo Ri Lee Han, Lorraine B. Ware, Julie A. Bastarache

Acute Respiratory Distress Syndrome (ARDS) is a common cause of acute respiratory failure. Despite extensive research in animal models, in which the syndrome is called Acute Lung Injury (ALI), no targeted therapy has been found to reduce its high mortality rate. Two major pathologic features of ARDS are loss of lung barrier integrity and activation of the Tissue Factor (TF) pathway of coagulation in the airspace. However, as an integral membrane protein TF also serves several non-coagulant functions including promotion of cell adhesion. All systemic anti-coagulants tested have failed to show clinical benefits in ARDS, with some trials of TF pathway inhibition showing increased mortality in ARDS patients. One explanation for these clinical results is that TF in the airspace is protective in ARDS. Supporting this concept, our previously published mouse work found that loss of alveolar epithelial cell TF caused increased loss of lung barrier integrity in models of ALI. As such, we hypothesize that epithelial TF is necessary for maintaining lung barrier integrity and that its overexpression will be protective in ALI. To determine whether supraphysiologic overexpression of TF in the lung can enhance barrier integrity we created a novel transgenic mouse in which TF was inducibly overexpressed in the lung epithelium (TFEpi+). Specifically, a human influenza hemagglutinin-tagged TF construct, driven by the CMV-TetO promoter and crossed with SPC-rtTA59 mice was used to produce inducible, lung epithelial-targeted TF overexpressing mice. High alveolar epithelial TF expression compared to wild-type littermates (WT) was confirmed through immunohistochemistry and western blot analysis after one week of doxycycline in drinking water. To induce ALI, mice were intranasally infected with 2000 colony forming units of Klebsiella pneumoniae or PBS. At 24-hours post infection, mice were euthanized, lung tissue was collected, and a bronchoalveolar lavage (BAL) was performed. Animal body weights were recorded pre-, and 24-hours post infection. BAL was analyzed to measure protein, clot time, and leukocyte influx. Lung tissue was utilized to calculate wet-to-dry weight ratios and bacterial burden. TFEpi+ mice infected with Klebsiella pneumonia showed lower BAL protein (385.63 vs 248.51 μg/ml; n=16; p=0.0019) and lung wet-to-dry weight ratios (5.24 vs 4.86; n=16; p=0.0560) compared to WT. However, weight loss, bacterial burden, BAL clot time, and BAL inflammatory cell counts did not differ between infected TFEpi+ and WT mice. These findings suggest that alveolar epithelial TF overexpression is protective for maintaining lung barrier integrity and a non-coagulate based mechanism, potentially linked to epithelial cell adhesion.

SYSTEMS ANALYSIS

Temporal modelling of the biofilm lifecycle (TMBL) establishes kinetic analysis of plate-based bacterial biofilm dynamics

Kyle Enriquez, W. Dale Plummer, Preston D. Neufer, Walter J. Chazin, William D. Dupont, Eric P. Skaar

Bacterial biofilms are critical to pathogenesis and infection, and are associated with rising rates of antimicrobial resistance. Since the presence of biofilms is correlated with worse clinical outcomes, the study of biofilms and biomass-associated bacterial communities is critical to advancing research into infectious diseases. There is a gap in knowledge surrounding the biofilm lifecycle as a continuum rather than a pre-defined step-by-step process. This underappreciation for kinetics and dynamics makes the study of biofilm formation, prevention, and destruction difficult to translate from bench to

bedside. To address this gap, this work employs a well-characterized crystal violet biomass accrual and planktonic cell density assay and expands statistical analysis to include kinetic information, in a protocol termed the TMBL (Temporal Mapping of the Biofilm Lifecycle) assay. TMBL's statistical framework allows for quantitative comparisons of distinct biofilm communities and growth conditions. This framework produces measurements across time, species, and media conditions in a 96-well format, is reactive to environmental changes, and is adaptable to disparate media conditions. Measurements from TMBL can reliably be condensed into response features that inform the time-dependent behavior of adherent biomass and planktonic cell populations. Staphylococcus aureus and Pseudomonas aeruginosa biofilms were grown in conditions of metal starvation in nutrient-rich and nutrient-poor media to demonstrate the rigor and translational potential of this strategy. Significant differences in single-species biofilm formation are seen in metal-deplete conditions as compared to their controls. In S. aureus, there is a significant increase in adhered biomass in the absence of calcium and a loss of biomass in response to zinc depletion. Taken together, these results suggest that kinetic analysis of biofilm by TMBL represents a statistically and biologically rigorous approach to studying the biofilm lifecycle as a semi-continuous process. In addition to current methods to study the impact of microbe and environmental factors on the biofilm lifecycle, this kinetic assay can inform biological discovery in microbial community development and evaluate the impact of exogenous factors on biofilm communities

A. CHEMICAL SYNTHESIS

A1. Application of umpolung amide synthesis toward the synthesis of feglymycin.
Preston Gourville, Jeffrey N. Johnston
The ubiquity of amide bonds in pharmaceuticals and natural products has driven the development of a wide array of methodologies for their formation. While a myriad of coupling reagents exists for conjoining an electrophilic carboxylic acid and a nucleophilic amine, these methods are mechanistically vulnerable to epimerization of the alpha carbon – losing its stereochemical integrity. Work in the Johnston lab has focused on developing methodology for Umpolung Amide Synthesis (UmAS), a mechanistically distinct amidation pathway that eliminates the potential for epimerization. UmAS utilizes a nucleophilic bromonitroalkane as an acyl anion equivalent and an *in situ* generated electrophilic *N*-haloamine to form an amide bond. We sought to apply this methodology to the total synthesis of the tridecapeptide feglymycin, a novel anti-HIV agent. Feglymycin's complex structure incorporates nine non-canonical arylglycinyl amino acids, which are mechanistically liable to epimerize under traditional amide bond forming reactions. Leveraging this methodology, we have applied UmAS to the total synthesis of the aryl glycine-rich tridecapeptide feglymycin. The latest results toward the total synthesis of feglymycin will be presented.

A2. Generating Non-Natural Methyltransferase Substrates via EnzHTP Optimized Halomethyltransferase AtHOL1.
Asher C Hollenbeak, BO Bachmann, QZ Shao, ZJ Yang
S-adenosyl-L-methionine(SAM)–dependent methyltransferases may be exploited to catalyze versatile alkylations to target small molecules (e.g. antibiotics). However, SAM analogs are unstable in solution. Halomethyltransferases (HMT) were found to catalyze the regeneration of SAM from Sadenosyl-homocysteine and halomethanes. If engineered to produce SAM analogs, HMTs could overcome SAM analog instability, allowing for versatile alkylation of antibiotics, greatly expanding the chemical diversity of antibiotic candidates from a single scaffold.

A3. Chemical Synthesis in Support of Chemical Biology.
Kwangho Kim
Chemical synthesis has been described as the central science as its practice provides access to chemical structures of known and/or designed function. Advances in human health especially benefits from synthesis in advancing basic science discoveries in chemical biology and new therapeutics in translational research. To support the chemical synthesis needs of investigators across campus the Vanderbilt Institute of Chemical Biology established a chemical synthesis core as part of its foundation in 2002. Provided in this review are examples of synthetic products, known and designed, produced in the core over the past ten years.

A4. Enantioselective Reduction of Vinylogous Nitroamines towards Non-Natural Amino Acids and Promising Therapeutics
Melanie Padalino and Jeffrey N. Johnston

β-Amino nitroalkanes are important precursors to a vast array of organic scaffolds such as α-amino amides, α-amino acids, and 1,2-diamines. Traditionally, the enantioselective reduction of vinylogous nitroamines towards these precursors required harsh conditions (hydrogen under pressure) and expensive metal catalysts such as iridium and rhodium. In recent years, there has been much focus on applying organocatalytic transfer hydrogenation to effect this transformation. We propose the use of MonoAMidinium (MAM) chiral proton catalysts to accelerate this reduction in good yield and excellent enantiomeric excess (*ee*). We have optimized this mild reduction and illustrate how the resulting *β*-amino nitroalkanes can be converted into a variety of useful products, particularly unnatural amino acids (UAAs), in enantiopure form. Progress towards a promising therapeutic for Duchenne Muscular Dystrophy (DMD) using this methodology will also be described. Overall, this work harnesses the power of enantioselective organocatalysis to access key enantioenriched scaffolds while expanding our tools for sustainable synthesis.

A5. Structural Studies on the Antimicrobial Marine Natural Product Chrysophaentin A and Congeners.
Gabriel Pongdee, Christopher Fullenkamp, Somnath Jana, Don Stec, Gary Sulikowski
Chrysophaentin A is an antimicrobial natural product isolated from the marine alga *C. taylori* in milligram quantity. Structurally, chrysophaentin A features a macrocyclic biaryl ether core incorporating two trisubstituted chloroalkenes at its periphery. Upon isolation fractions of chyrsophaentin A surprisingly showed circular dichroism (CD) active indicating optical activity (enantiomerically enriched). In addition, NMR analysis of chrysophaentin A analogs, obtained by chemical synthesis, showed temperature dependent behavior indicating dynamic conformational properties often associated with atropisomerism. Variable temperature NMR analysis of Mosher ester derivatives support this assumption and indicate a barrier of approximately 15 Kcal of two interconverting conformers. Work toward derivatives incorporating groups aimed at raising the barrier of this interconversion leading to separable enanatiomers will be described.

A6. Generating Novel Everninomicin Analogs Using a KASIII-type Acyltransferase from *Micromonospora carbonacea*
Jennifer Wurm, Audrey Yniguez-Gutierrez, Jordan Froese, Nicolas Rosenthal, and Brian Bachmann
Orthosomycins are potent oligosaccharides that inhibit bacterial protein synthesis with a unique target binding site hypothesized to contribute to their efficacy against multidrug-resistant bacteria. While many clinically used antibiotics that also target the ribosome bind at the peptidyl transferase center, orthosomycins bind at a site 50 angstroms away in an understudied region. Previous studies began to investigate the

ribosomal interactions between binding site residues and everninomicin, an orthosomycin which advanced to phase III clinical trials, but the drug was ultimately discontinued due to aggregation upon intravenous drug formulation. Despite everninomicin's unique binding site and potency against drug-resistant bacteria, the difficulty of synthesizing everninomicin analogs has hindered development of an optimized version for drug candidacy. With chemical synthesis of the full molecule requiring over 130 steps, our lab developed an in vitro method to create everninomicin analogs by utilizing the biosynthetic machinery of its producing strain Micromonospora carbonacea var. aurantiaca. We recently optimized large-scale fermentation of our engineered M. carbonacea strain that produces a precursor everninomicin heptasaccharide metabolite (EVQ) lacking its terminal dichloroisoeverninic acid (DCIE) ring. Optimization of EVQ isolation and purification from an 8-liter fermentation then produced a yield of 90 milligrams of purified EVQ. Using EVQ as a building scaffold for analog synthesis, we chemically synthesized a small library of aryl DCIE-ring derivates to attach to EVQ and developed a shortened synthetic route for their preparation. To execute the aryl transfer onto to EVQ, we identified, expressed, and purified a ketoacyl synthase-III (KASIII)-like enzyme in the gene cluster (EvdD1) with homology to KASIII enzymes capable of acting as acyltransferases. Combining EvdD1, EVQ, and DCIE-ring derivates in vitro, we successfully generated four novel everninomicin analogs and validated a ribosome inhibition assay to assess their activity. Through synthesis of additional everninomicin analogs and subsequent activity analysis, we anticipate to determine the effect that DCIE-ring alterations have on specific ribosomal interactions, providing valuable insight on target engagement in the process of developing an optimized drug candidate.

B. SYSTEMS ANALYSIS

B1. Effect of Supercoil Handedness on Intermolecular Strand Passage Catalyzed by Gyrase

Jillian F. Armenia, Neil Osheroff

Most bacteria encode two type II topoisomerases, gyrase and topoisomerase IV, that resolve topological problems associated with DNA. Topoisomerase IV mainly performs intermolecular DNA strand passage reactions (catenation and decatenation) while gyrase functions ahead of DNA tracking machinery to remove intramolecular positive supercoils. Some bacteria, including those from the *Mycobacterium* genus, encode gyrase as their sole type II topoisomerase, meaning that gyrase likely catalyzes both intra- and intermolecular strand passage reactions. How gyrase is able to differentiate between topological outcomes *in vivo* remains an open question. One possibility is that gyrase preferentially catalyzes intermolecular strand passage on substrates of a specific supercoil handedness. To test this hypothesis, we examined the ability of gyrase from *Bacillus anthracis* to catalyze catenation of positively supercoiled [(+)SC], negatively supercoiled [(-)SC], and relaxed DNA. We found that wild-type *B. anthracis* gyrase is a poor catenase, but does recognize topology. Gyrase preferentially catenates (+)SC and relaxed DNA over (-)SC DNA. We also sought to test the effect of DNA strand wrapping by gyrase on catenation of differently supercoiled substrates. Without the GyrA-box motif in the C-terminus of GyrA, gyrase loses the ability to strand-wrap, but can distinguish supercoil handedness during DNA relaxation. To test whether this applies to intermolecular reactions, we examined the ability of a *B. anthracis* GyrA-box mutant gyrase to catenate (+)SC, (-)SC, and relaxed DNA. Without strand wrapping, *B. anthracis* gyrase became a more efficient catenase and maintained the ability to differentiate supercoil handedness during catenation. Because gyrase acts mainly to remove torsional stress on (+)SC DNA, strand wrapping acts as a mechanism to prevent aberrant catenation ahead of replication or transcription machinery. Work is ongoing to examine the effect of topology on intermolecular strand passage reactions catalyzed by gyrase from species which lack a topoisomerase IV, such as *Mycobacterium tuberculosis*.

B2. Enhanced LC-MS/MS Proteomics Approaches to Elucidate the Unfolded Protein Response (UPR) Proteostasis Remodeling

Lea A. Barny and Lars Plate

The proper folding of secretory proteins is vital to a variety of cellular and organism functions. An accumulation of non-native protein conformations in the endoplasmic reticulum (ER), termed 'ER stress,' can result from numerous insults to the ER, including an increased abundance of aggregated and misfolded proteins, changes in redox state, oxidative stress, and calcium dysregulation, etc. To restore ER proteostasis and prevent the trafficking of non-native protein conformations to secretory environments, cells have evolved a highly conserved tripartite signal transduction pathway known as the Unfolded Protein Response (UPR). The three branches of the UPR: ATF6, IRE1, and PERK, upregulate the synthesis of a trafficking, and degradation of nascent proteins to restore ER proteostasis. Importantly, current transcriptomics and western blotting approaches to study the UPR lack the ability to disentangle the functional consequences of the three arms of the response. However, liquid chromatography- mass spectrometry proteomics provides the ability to quantitatively measure numerous proteostasis factors reproducibly. We employed two untargeted proteomics methodologies, fractionated (MudPIT)-data dependent (DDA) and data independent acquisition (DIA), to evaluate the depth of UPR target proteins detected between the two methods. Data-independent acquisition resulted in a greater diversity and number of proteins quantified. Lastly, a targeted method was determined to be able to detect UPR activation, branch specifically, allowing for the high throughput screening of multiple UPR target proteins in a single mass spectrometry analysis.

B3. Time-Resolved Host-Pathogen Interactomics of SARS-CoV-2 Non-structural Protein 15

Crissey Cameron, R. Mason Clark, Runze Jiang, Adam M. Metts, LaToya D, Skaggs and Lars Plate

Viral infection, specifically severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), poses an immense global health burden. To combat current and future viral outbreaks, it is essential to develop informed antiviral therapies. Coronaviruses rely on virus-host protein-protein interactions (PPIs) to disrupt host cellular processes and prevent a host antiviral response that can be disturbed with host-targeted therapeutics. Because viral infection is a tightly regulated, temporal process, it is important to elucidate the timing of host-pathogen interactions to be able to target critical interactions in the development of therapeutics. Temporal resolution of PPIs can be accomplished using a destabilized dihydrofolate reductase (dDHFR) fusion that is constitutively degraded by the proteosome. When a trimethoprim (TMP) probe is applied, the fusion protein is stabilized and accumulates in the cell. This provides a way to synchronize the translation of a population of protein and label it for future enrichment and proteomics analysis. We have established the methodology to be able to resolve the timing of PPIs using a dDHFR-YFP fusion as a model and applied this methodology to SARS-CoV-2 non-structural proteins (nsps) responsible for activation of the host immune response. This project has given insight into the sequence of nsp15 interactions with host proteins as well as how nsp15 prevents IRF3 nuclear localization as part of the host immune response.

B4. Temporal modelling of the biofilm lifecycle (TMBL) establishes kinetic analysis of plate-based bacterial biofilm dynamics

Kyle T. Enriquez, MS, W. Dale Plummerd, Preston D. Neufere, Walter J. Chazin, Ph.D., William D. Dupont, PhD, Eric. P. Skaar, PhD MPH

Bacterial biofilms are critical to pathogenesis and infection, and are associated with rising rates of antimicrobial resistance. Since the presence of biofilms is correlated with worse clinical outcomes, the study of biofilms and biomass-associated

bacterial communities is critical to advancing research into infectious diseases. There is a gap in knowledge surrounding the biofilm lifecycle as a continuum rather than a pre-defined step-by-step process. This underappreciation for kinetics and dynamics makes the study of biofilm formation, prevention, and destruction difficult to translate from bench to bedside. To address this gap, this work employs a well-characterized crystal violet biomass accrual and planktonic cell density assay and expands statistical analysis to include kinetic information, in a protocol termed the TMBL (Temporal Mapping of the Biofilm Lifecycle) assay. TMBL's statistical framework allows for quantitativecomparisons of distinct biofilm communities and growth conditions. This framework producesmeasurements across time, species, and media conditions in a 96-well format, is reactive toenvironmental changes, and is adaptable to disparate media conditions. Measurements from TMBL can reliably be condensed into response features that inform the time-dependent behavior of adherent biomass and planktonic cell populations. *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms were grown in conditions of metal starvation in nutrient-rich and nutrient-poor media to demonstrate the rigor and translational potential of this strategy. Significant differences in single-species biofilm formation are seen in metal-deplete conditions as compared to their controls. In S. aureus, there is a significant increase in adhered biomass in the absence of calcium and a loss of biomass in response to zinc depletion. Taken together, these results suggest that kinetic analysis of biofilm by TMBL represents a statistically and biologically rigorous approach to studying the biofilm lifecycle as a semi-continuous process. In addition to current methods to study the impact of microbe and environmental factors on the biofilm lifecycle, this kinetic assay can inform biological discovery in microbial community development and evaluate the impact of exogenous factors on biofilm communities.

B5. In situ lipidomics of Staphylococcus aureus bone infection using MALDI imaging mass spectrometry.

Christopher J. Good, Casey E. Butrico, Elizabeth K. Neumann, Madeline E. Colley, Katherine N. Gibson-Corley, Lukasz G. Migas, Raf Van de Plas5, James E. Cassat, Jeffrey M. Spraggins, and Richard M. Caprioli

Osteomyelitis is a debilitating infection of bone, usually caused by *Staphylococcus aureus*, and carries significant patient morbidity due to the high rate of treatment failure. Bacterial colonization of bone and bone marrow yields a sequestered inflammatory tissue lesion known as an abscess. This host-pathogen interface is highly spatially defined and yields dynamic molecular interactions that drive the progression of *S. aureus* osteomyelitis. Technologies such as matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) can be employed to facilitate the discovery of spatially resolved chemical information in biological tissue samples to help elucidate the complex molecular processes underlying pathology. In this work, a multimodal MALDI IMS-microscopy platform was leveraged to define the lipid composition of fracture-stimulated tissue and bone marrow abscesses. The periosteum, or the outer membrane of the hard bone, was much larger compared to mock-infected tissue alluding to an inflammatory reaction, and this tissue layer was rich with sphingomyelins (SMs) and phosphatidylcholines (PCs). Ether PCs (e.g. $[PC\ O\text{-}(32:0) + K]^+$, $[PC\ O\text{-}(34:2) + K]^+$) and sphingolipids (e.g. $[SM(34:1);O2 + K]^+$, $[SM(42:3);O2 + K]^+$) were more abundant in mature abscesses compared to its contiguous bone marrow and separate mock-infected tissue. On the contrary, arachidonoyl glycerophospholipids (e.g. $[PI(18:0\_20:4) - H]^-$, $[PE(18:0\_20:4) - H]^-$) were depleted in the abscesses suggesting their programmed decomposition for arachidonic acid-mediated cell signaling (PI=phosphatidylinositol, PE=phosphatidylethanolamine). Fluorescence microscopy and targeted data mining helped discover lipid signatures associated with cells on the outer border of the necrotic regions. Based on these lipid distributions, we believe the abscess border consists of unique and viable neutrophils, macrophages, and fibroblasts that are resolving inflammation by storing arachidonic acid and actively digesting lipoproteins and other cellular debris.

B6. Disulfide Bond Coordination During Thyroglobulin Folding

Jake Hermanson, Charles Sanders

Protein function is dependent on the correct folding of polypeptides into their native conformations. In a cellular environment, molecular chaperones, folding, and degradation factors, which together constitute the proteostasis network, either help correct the misfolding or target terminally misfolded proteins for degradation. However, missense mutations can prevent a protein from adopting its correct conformation. Failure by the proteostasis network to correct the protein folding defects can give rise to a variety of protein misfolding mutations. My project investigates the proteostasis pathways surrounding the secreted thyroid prohormone thyroglobulin (Tg) which has misfolding mutations that limit secretion. My overarching goal is to develop a rapid time-resolved interactomics methodology to track the interactions with chaperones and folding machinery during the early biogenesis steps. For this purpose, I constructed a Tg-HaloTag fusion protein and using biotin HaloTag ligands for rapid pulse-labeling of a Tg population to capture and track their time-resolved proteostasis interactions at high resolution. One application is to understand how disulfide bonds form and what molecular chaperones ensure correct formation. The Tg native structure contains 60 disulfide bonds and their correct pairing requires engagement of a series of protein disulfide isomerase enzymes. Following the formation of disulfide bonds can be used as a proxy to help understand how Tg folds into the native conformation. This will lead to insight into how disulfide bonds form in secreted proteins, which is important in many pharmaceuticals.

B7. Glial Phagocytosis of Brain Neurons Mediated by FMRP-Dependent JNK signaling for Filamin Control of the Actin Cytoskeleton

Rincon Jagarlamudi, Kendal Broadie

Juvenile brain circuit remodeling is a crucial developmental process that is disrupted in a number of different neurological disease states, including Fragile X syndrome (FXS); the

leading heritable cause of intellectual disability (ID) and autism spectrum disorder (ASD). Recent work suggests that the developmental clearance of neurons during circuit remodeling requires neuron-to-glia signaling that drives glial phagocytosis. We study these events in the *Drosophila* brain PDF clock circuit, which contains the developmentally transient PDF-Tri neurons. We have previously shown that the Fragile X Mental Retardation Protein (FMRP) is required in neurons to activate and recruit glia for the phagocytosis and developmental clearance of these PDF-Tri neurons.Neuronal Fragile X Mental Retardation Protein (FMRP)-dependent signaling activates the glial engulfment receptor Draper (mammalian Megf10/Jedi) for the phagocytic clearance of developmentally transient PDF-Tri neurons in *Drosophila.* We have now found that glial Basket (mammalian Jun N-terminal kinase; JNK) signaling and the downstream AP-1 transcriptional pathway are required for the clearance of PDF-Tri neurons. We found that targeted neuronal FMRP and glial Draper RNAi mutants exhibit reduced Basket/JNK translocation into glia nuclei, indicating a disruption in JNK signaling to AP-1 transcriptional control. We discovered this JNK/AP-1 signaling pathway drives the glial expression of Cheerio (mammalian filamin A; FLNA), an F-actin cross-linking protein, which is required for the PDF-Tri neuron clearance. Null *cheerio* mutants and glia-targeted *cheerio* RNAi similarly prevent removal of the normally developmentally transient PDF-Tri neurons. We also discovered FMRP genetically interacts with Cheerio to impact glia at a cytoarchitectural and functional level. Transheterozygous animals with only one copy of the FMRP and Cheerio genes display an impaired glial actin cytoskeleton and complete blockade of PDF-Tri neuron removal. We discovered that the glial processes in both FMRP and Cheerio mutants have a diminished ability to infiltrate the PDF-Tri region and produce phagocytic markers. We found that neuronal FMRP drives Basket/JNK signaling principally in ensheathing glia, which use the Cheerio/FLNA actin crosslinker to migrate to and clear the PDF-Tri neurons. Taken together, we conclude that neuronal FMRP drives glial Basket/JNK signaling to induce AP-1 transcription of Cheerio/FLNA to modulate the glial F-actin cytoskeleton to enable glial phagocytosis of the PDF-Tri neurons. Our work identifying structural actin defects in Fragile X and Cheerio mutants contributes to the hypothesis that downstream down-regulation of cheerio in Fragile X animals prevents glial process extension, via their actin cytoskeleton. This clearance pathway has important implications for the study of Fragile X syndrome, as it provides a new cellular mechanism for the disease state and new molecular targets for the development of new therapeutic treatments.

B8. Evaluating the molecular biophysical basis for HIKESHI Hypomyelinating Leukodystrophy (HHL) variant destabilization

Christina Mercado, Drew Neufer, Joe DeCorte, Ben Brown, Jens Meiler, Kaitlyn Ledwitch

HIKESHI Hypomyelinating Leukodystrophy (HHL) is a lethal pediatric neurodegenerative disorder in which children suffer from developmental delay, spasticity, and microencephaly. The normal HIKESHI protein dimer functions as a nuclear mediator and shuttles Hsp70s to protect cells against heat-induced damage. HHL variants V54L, P78S, and C4S are localized to the N-terminal binding domain (NTBD) of HIKESHI, and while there is a crystal structure of wild-type HIKESHI, we lack atomic details describing the molecular biophysical basis for HHL. In this work, we assessed the predicted thermodynamic stability of each HHL mutant using the Cartesian ΔΔG application in Rosetta. We follow this up with an integrated structural biology approach to assess HHL variant destabilization in atomic detail using deep-learning / machine-learning tools like AlphaFold 2 (AF2) and RoseTTAFold combined with nanoDSF, solution NMR, and X-ray crystallography. Preliminary data shows initial crystal screening conditions, a nanoDSF thermogram with a melting temperature of 55°C for wild-type protein, and Rosetta ΔΔG calculations of HIKESHI variants, which suggest destabilization in both NTBDs. We apply this framework to drive HHL drug discovery efforts using ultra-large library screening (ULLS) with AF2-enabled modeling of HHL variants to identify and screen drug-binding pockets for mutant-specific structural stabilizers.

B9. Maintenance of organismal and cellular homeostasis by an ancient zinc metallochaperone

Murdoch C.C., Weiss A., Edmonds K.A., Jordan M.R., Perera Y.R., Petoletti A.M., Monteith A.J., Beavers W.N., Munneke M.J., Drury S. L., Krystofiak E.S., Thalluri K., Wu H., Kruse A.R.S., Dimarchi R.D., Caprioli R.M., Spraggins J.M., Chazin W.J., Giedroc D.P., and Skaar E.P.

Zinc (Zn) is an essential micronutrient and cofactor for up to 10% of proteins in living organisms. During Zn limitation, specialized enzymes called metallochaperones are predicted to facilitate Zn transfer to metalloproteins. This function has been putatively assigned to G3E GTPase COG0523 proteins, yet no Zn metallochaperone has been experimentally identified in any organism. Here, we functionally characterize a family of COG0523 proteins that is conserved across vertebrates. We identify Zn metalloprotease METAP1 as a COG0523 client, leading to the redesignation of this group of COG0523 proteins as Zn regulated GTPase metalloprotein activator (ZNG1) family. Using biochemical, genetic, structural, and pharmacological approaches across evolutionarily divergent models, including zebrafish and mice, we demonstrate a critical role for ZNG1 proteins in regulating cellular Zn homeostasis. Collectively, these data reveal the existence of a family of Zn metallochaperones and place ZNG1 at the center of a new paradigm for intracellular Zn trafficking.

B10. Characterizing effects of coronavirus nonstructural protein nsp2:4EHP/GIGYF2 interaction.

Brynn Roman, Lars Plate

Nonstructural proteins (nsps) play a crucial role setting up RNA viral replication in the infected host cell. Exerting control

over host translation machinery is necessary during the viral life cycle to slow endogenous host protein synthesis while promoting viral protein translation. However, the underlying mechanisms by which viral nsps regulate protein translation are not well-understood. SARS-CoV-1 nsp2 has been identified as a viral protein that interacts with host translation repressors 4EHP and GIGYF2. SARS-CoV-2 nsp2 does not strongly interact with 4EHP or GIGYF2, despite the nsp2 homologs having a sequence similarity of 79.1%. As 4EHP and GIGYF2 are translational repressors, it is important to determine the effects of the nsp2 homologs on the host proteome and host translation. To this end, we utilized tandem mass spectrometry to analyze differences in the host proteome in the presence of SARS-CoV-1 and SARS-CoV-2 nsp2. Significant differences in both host protein abundances and translation rates affected by the presence of each homolog were observed, indicating the SARS-CoV-1 and SARS-CoV-2 nsp2 are targeting different host proteins. In addition to characterizing effects of nsp2 on the host proteome, we have identified the general region of nsp2 responsible for the difference in 4EHP/GIGYF2 interaction strength using SARS-CoV-1 nsp2 truncations and SARS-CoV and SARS-CoV-2 chimeras. Upon identifying the 4EHP/GIGYF2 binding region of nsp2, nsp2 chimeraswith a mutated binding region were utilized to determine effects of SARS-CoV-1 andSARS-CoV-2 nsp2 that can be attributed to the nsp2:4EHP/GIGYF2 interaction

B11. Lipidomic characterization of mucosal and submucosal regions along the zebrafish gastrointestinal tract using High-Resolution MALDI Imaging mass spectrometry.

Jacquelyn Spathies, Caitlin C. Murdoch, Eric P. Skaar, Jeffrey M. Spraggins

The gastrointestinal tract (GI) of adult zebrafish contains heterogeneous cell populations such as mucin-secreting goblet cells that help create a mucosal barrier similar to humans. This barrier is the first line of defense against infection and is heavily impacted during inflammation and injury. Molecular imaging technologies, such as imaging mass spectrometry (IMS), can be co-registered with microscopy data to determine the molecular profiles of specific tissue regions and cell types along the zebrafish GI tract, including the mucus layer. Herrin, we characterize the lipidomic profile of the mucosal and submucosal regions along the zebrafish GI tract based on IMS, fluorescence, and stained microscopy.

B12. Multi-omics analysis of Nrf2 and αB-crystallin mutant zebrafish lenses under oxidative stress

Sarah R. Zelle, Jinhee Park, Samantha MacGavin, Laurie Niederbrach, Kevin L. Schey, Hassane S. Mchaourab

Cataracts are an opacification of the eye lens caused by biochemical and physiological dysfunction. Oxidative stress is thought to be important in age-related cataract (ARC) development, but how oxidative damage in the lens leads to cataract formation is unclear. Previous results also suggest that the oxidative stress response pathway can alter the proteostatic balance within the lens. I hypothesize that ARC formation is caused by proteomic changes that result from an age-related compromised oxidative stress response and altered proteostatic state of the lens. Therefore, multi-omic analyses of the oxidative stress response in the lens are needed to better understand the oxidative stress response pathway *in vivo*.

C. MOLECULAR DISCOVERY

C1. Machine-learning guided discovery of bioactive compounds.

Christine Ancajas and Allison Walker

Microorganisms are known producers of natural products which have been exploited to be used as antibiotics, antifungals, and anticancer agents. However, many of these compounds in clinical use have been compromised by the rapid emergence of antimicrobial-resistance. Moreover, an additional challenge in drug discovery is the time-consuming process and rediscovery of known molecules. To address this roadblock, a machine learning bioinformatics approach was utilized to prioritize discovery of novel compounds with useful functions. The algorithm predicts biological activity from biosynthetic gene clusters and other genetic descriptors such as dehydrogenases involved in formations of pyrrole and nitro sugar-containing products. Bacterial genomes from a library were screened through the machine learning platform and promising candidates such as *Streptomyces rimosus* were studied. Metabolites and their biosynthesis were analyzed and elucidated using isotope labeling, high-performance liquid chromatography, mass spectrometry, and NMR. The results of the study suggest that Streptomycetes and other microbes continue to be a rich source for novel bioactive compounds and that the molecular features associated with the dehydrogenases have promising roles as handles for activity.

C2. Enhancing Bioactivity Prediction of Natural Products using 3D Structural Data and Machine Learning.

Raven Dean, Allison Walker

Natural products have been historically significant for drug discovery, with nearly two-thirds of small-molecule drugs approved between 1981 and 2019 derived from natural products. Machine learning has proven to be an effective tool for predicting the properties of natural products using 2D coordinate-based descriptors. However, the potential impact of 3D structural data as descriptors for machine learning algorithms in predicting the bioactivity of natural products poses an exciting frontier for research. In this project, we propose a featurization method supported by 3D structural data to improve the accuracy of bioactivity predictions for natural products. Our objectives are to develop and test a featurization method for the message passing neural network (MPNN) machine learning model that incorporates 3D data as a feature of the molecules in the datasets. We conducted range tests to determine the optimal range of 3D data for highest overall accuracy, finding that biologically active datasets showed an average improvement of -34.8%, while biologically irrelevant datasets showed an average improvement of -45.6%. The most positive percent difference of 8.8% was observed at 2.3 Å distance threshold. Additionally, the datasets were analyzed to identify the natural products and natural-product-like molecules contained within them, which further support the specificity of our featurization method towards natural products. The results of this study demonstrate the potential of incorporating 3D structural data in machine learning algorithms to significantly enhance the prediction of bioactivity machine learning in chemistry and drug discovery and highlights the importance of considering 3D structural information to improve predictive accuracy in natural product research.

C3. The Vanderbilt University High Throughput Screening Core Facility, Vanderbilt Institute of Chemical Biology: Harnessing the Power of Chemistry to Improve Human Health.

Thomas Hasaka, Ph.D.

The High Throughput Screening (HTS) Core Laboratory provides screening-based services and resources to aide research investigators in the identification and investigation of molecular probes/compounds for basic research and pharmacological discovery. We offer a highly dynamic environment that utilizes industry-standard practices and cutting-edge technologies for biological screening. The HTS facility is equipped to guide investigators through the entire screening and drug discovery process, from assay conception to lead optimization. In the HTS lab, we serve the basic research needs of scientific investigators by providing full-service access to state-of-the-art instrumentation and software, distribution of compound or genetic libraries and informatics solutions. Most assays are amenable to HTS including biochemical (enzymatic, protein-protein interaction), cell-based (viability, real-time kinetic, imaging), 3D models (tumorspheres, spheroids, organoids) and model organisms (zebrafish, C. Elegans, Drosophila). Transferring these assays to a multi-well plate-based format and automated techniques for experimental execution can provide several advantages including improved reproducibility and quality, increased throughput and the ability to test/screen many different conditions in a single experiment. The HTS facility offers a broad variety of chemical collections that would be of interest to many research investigators. For example, the core laboratory houses collections containing structurally diverse and biologically active compounds, FDA approved drugs for cancer and other diseases, compounds that have a history of use in human clinical trials, natural products, ion channel targeting compounds and kinase inhibitors.

C4. Development of the first vascular-specific K_{ATP} channel inhibitor for the treatment of patent ductus arteriosus in newborns.

KJ Li, Samantha J. McClenahan, Craig W. Lindsley, Elaine L. Shelton, 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. 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 (K_{ATP}) 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 K_{ATP} 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 IC_{50} of approximately 100 nM and is highly selective for Kir6.1/SUR2B over Kir6.2/SUR1 and several other Kir channels. Understanding the molecular basis of VU0542270 selectivity is critical for understanding its mechanism of action, prompting us to leverage recently developed cryo-EM structures of Kir6.1/SUR2B and molecular docking techniques toward this goal. Preliminary modeling data and functional characterization of mutants carrying putative binding site mutations suggest that VU0542270 and glibenclamide interact with distinct yet partially overlapping binding sites in SUR2. Ongoing experiments utilizing classical radioligand binding assays and mutagenesis are being performed to determine the molecular binding site of VU0542270 with greater detail. We anticipate these studies will reveal VU0542270 mechanism of action and help inform the next generation of potent and highly selective Kir6.1/SUR2B K_{ATP} channel inhibitors.

C5. High-Throughput Screening by Panoptic Kinetic Imaging Plate Reader

Dehui Mi, Emily Days, Liangping Li, David R. Baughman, Joshua A. Bauer.

The Vanderbilt High-Throughput Screening (VHTS) core provides screening-based services to aid research investigators in the identification and investigation of new compounds for basic research and pharmacological discovery. In a highly dynamic environment that utilizes industry standard practices and novel technologies for biological screening, investigators are guided through the drug discovery process from assay conception to lead compound identification. Both walk-up or full-service access to state-of-the-art instrumentation, distribution of compound libraries, and informatics solutions are available. Since our prior drug discovery targets are mainly G-protein-coupled receptors (GPCRs) and ion channels, we use kinetic imaging plate readers to perform various assays specifically probing these targets. The VHTS Core is equipped with two Panoptic kinetic imaging plate readers developed by WaveFront Biosciences. Their broad application use is enabled by a combination of outstanding optics and automation friendly on-board liquid handling. The Panoptic offers great flexibility including 10-excitation-5-emission filter sets for fluorescence as well as detection of luminescence. It also has low image distortion, the finest kinetics (30 Hz), and a thermoelectrically cooled (TEC) highly sensitive CCD camera for high temporal resolution while maintaining spatial resolution. The multi-nest liquid handler of Panoptic makes multi-injection (e.g. compound, ligand, control-adds) kinetic assays possible, with on-board tip exchange and tip washing and enhanced temperature control from ambient to 42 °C. The accompanying Waveguide software extends the convenience for data acquisition, annotation/curation, analysis, and data viewing in a high-throughput manner. Over the past decade, VHTS has provided user training, assay development, small and large library screening, and data analysis to a wide Vanderbilt user group. Inhouse instrument maintenance and informatics support plus consumable supply, ensure smooth operation of the Panoptic. Panoptic related research spans various applications such as calcium flux, thallium flux, membrane potential, neurotransmitter transport, cAMP GloSensor, and BRET assays, to name a few. Exemplary instrument features and projects will be highlighted. Prosperous publications from Panoptic use have made it one of the pillar technologies of the VHTS.

C6. *Clostridioides difficile* scavenges an unconventional nucleobase during colonization of the gut

Matthew J. Munneke, M. Wade Calcutt, Valérie de Crécy-Lagard, & Eric P. Skaar

Clostridioides difficile is the leading cause of nosocomial infectious diarrhea, and faces competition for nutrients from both the microbiota and immune system during infection. Amongst these nutrients are nucleobases, and nucleobase metabolism is critical for pathogenesis. We found that *C. difficile* can utilize 4-thiouracil (4-TU) as a uracil source in the vertebrate gut. The metabolism of 4-TU is mediated by proteins containing domain of unknown function 523 (DUF523). *C. difficile* encodes for two DUF523 paralogs, one of which is required for growth in the presence of 4-TU and which we have named, TudS. Additionally, *Escherichia coli* lacks a DUF523 homolog and 4-TU inhibits growth. We found that heterologous expression of *C. difficile tudS* is sufficient to restore growth of *E. coli* in 4-TU. Due to the structural similarity between 4-TU and uracil, we hypothesized that 4-TU is toxic because of misincorporation into RNA. Indeed, we discovered that 4-TU is incorporated into RNA in the absence of TudS. To identify additional components involved in 4-TU metabolism, we conducted a genetic selection in *E. coli* in the presence of 4-TU. We discovered that mutations in uracil phosphoribosyltransferase, a component of the uracil salvage pathway, are sufficient to overcome 4-TU toxicity and prevent 4-TU incorporation into RNA. These data suggest that 4-TU hijacks the uracil salvage pathway, and *C. difficile* exploits this through TudS conversion of 4-TU to uracil in the first step of 4-TU salvage. We hypothesize that this metabolic mechanism gives *C. difficile* an advantage in the competitive gut environment.

C7.

Abiodun Samuel Oyedele,

The key tool to the advancement in drug discovery has been explorations of genes connected to specialized secondary metabolites. About fifty percent of the approved drugs are known to be made of natural products and their derivatives. The Beta-lactam family has been at the forefront of the antibiotic drugs of which nocardicin has stood out due to its potency against gram-negative bacteria and partial escape from β-lactamase resistance. Here, I will be reporting the use of a few numbers of machine learning tools such as antiSMASH and cblaster in linking the nocardicins A and its analogs to

their cognate biosynthetic gene clusters (BGCs) and the predicted antibiotic activity. Several metabolomic approaches such as mass spectroscopic and nuclear magnetic resonance techniques would be employed in the empirical validation to networking chemotype to genotype

C8. Identification of Commercially Unavailable Oxazolomycin D Biosynthetic Gene Cluster and Subsequent Isolation

Kathryn E. Penton, Hannah Thirman, Madeline Hayes, Dr. Jonathan Irish, Dr. Brian O. Bachmann

While natural products continue to hold clinical relevance, discovery of novel compounds is decreasing due to high rates of rediscovery and reliable and reproducible ways to unlock potentially bioactive biosynthetic gene clusters (BGCs). By applying genome analysis tools to the excess of newly sequenced genomes, researchers can begin to characterize and link experimentally isolated products to their respective gene clusters in addition to unearthing BGCs containing diagnostic genes of interest. Our lab has shown the addition of distinct stimuli modulates compound expression while comparative metabolomics strategies can be used to demonstrate metabolite production and regulation trends. This workflow was applied in the search of novel analogs belonging to the small family of oxazolomycins, a β-lactone, γ-lactam containing hybrid NRPS/Type I PKS molecule. While β-lactone functional compounds have led to various clinical applications such as antimicrobial and anticancer agents; little is known about the oxazolomycins biological applications due in part to the fact that they are commercially unavailable. Utilizing genome mining, we have successfully identified a β-lactone containing hybrid NRPS/Type I PKS gene cluster in an unknown oxazolomycin producing strain, *Streptomyces libani* subsp. *rufus*. After applying a novel solid phase culturing technique which leverages the natural growth conditions of actinomycetes while stimulating the actinomycete genome through the addition of competing organisms, a target compound was identified. Through HPLC-MS, liquid chromatography, and two-dimensional NMR techniques this target compound was isolated and identified as Oxazolomycin D. While this is a previously discovered member of the oxazolomycin family, the gene cluster has yet to be identified and little is known in terms of bioactivity. In addition to linking this natural product to its gene cluster, we have observed Oxazolomycin D expression across four biological stimuli observing the highest levels of production when cultured alongside the mycolic acid containing bacteria *Tsukamurella pulmonis*. Subsequent experiments are ongoing to determine bioactivity of this underexplored member of the oxazolomycin class of compounds.

C9. Pre-isolation assessment of bioactivity for genome mining workflow optimization by Multiplexed Activity Metabolomics

Henry A. M. Schares**,** Joseph A. Balsamo, Jordan T. Froese, Benjamin J. Reisman, Kathryn E. Penton, Sierra M. Barone, Jonathan M. Irish, Paul B. Ferrell & Brian O. Bachmann

Natural product discovery by genome mining enabled the natural product community to flip the historical natural product discovery paradigm by making it possible to identify metabolites with structural characteristics of interest prior to isolation and assessment of activity. This streamlined the discovery process and enabled biasing discovery efforts towards novelty, leading to the accelerated discovery of many novel natural products. However, despite having knowledge of structural characteristics prior to isolation, clinically relevant bioactivity is not guaranteed for novel metabolites that required significant time and resources to isolate and structurally elucidate, undermining the advantages of the streamlined and focused aspects of genome mining. Our group has addressed this gap by connecting genome mining and bioactivity through the application of Multiplexed Activity Metabolomics (MAM), our high-throughput multi-phenotypic fluorescence cytometry assay. MAM uses chromatographic well plate arrays of crude extracts generated by HPLC/MS and fluorescent cell barcoding to generate a 6-immunomarker activity profile for each well of the assay plate that maps directly to UV and MS data of the well contents. Beneficially, all wells and internal controls are pooled into a single cytometric tube, significantly reducing antibody reagent consumption, increasing sample throughput, and decreasing well assay covariance. Adding MAM to the genome mining workflow enables efficient identification of metabolites of interest and expansive assessment of their bioactivity prior to isolation. This aids prioritization of active metabolites for isolation and reduces time and resources spent isolating inactive metabolites. Here we show the expansion of the genome mining workflow via MAM identified target metabolites that elicited perturbation of cell homeostasis, as well as identied metabolites of interest that did not elicit any bioactivity prompting reprioritization of effort. Implementing MAM into the genome mining workflow efficiently addresses the risk-reward shortcomings of natural product discovery via genome mining, streamlines the subsequent isolation process, and the high content nature of the MAM activity profiles can provide early mechanistic insights.

C10. Heterogeneity in Rocaglate Bioactivity Revealed Using Single Cell Approaches

Hannah Thirman, Jonathon Irish

Rocaglates are a family of compounds with promising therapeutic potential for the treatment of cancer and infectious diseases. Their reported molecular targets are the prohibitins, multi-functional membrane-localized proteins, and eIF4A, an RNA helicase implicated in translation initiation. However, the resulting cellular mechanisms impacted by rocaglate modulation, and the structural moieties driving them are not fully understood. To address this gap, the goal of this study is to dissect the relationship between rocaglate structure and cellular mechanism using phospho-specific flow-cytometry, a method enabling a range of cellular functions to be simultaneously quantified at the single cell level. An initial group of forty rocaglates that fall into four main structural subgroups were used to stimulate MV-4-11 cells, a model leukemia cell line. The underlying signaling response was quantified based on expression levels of 11 phospho-proteins governing cell growth, translation, and death. Through this approach, I revealed that the rocaglate structural subgroups have distinct impacts on MV-4-11 cells. When comparing the expression of each phosphoprotein measured, I discovered that

there was the most variation in subclass specific expression of γH2AX, a readout of genomic stress, and p-4EBP1, an effector downstream of mTOR. This subclass-specific divergence in γH2AX and p-4EBP1 expression replicated in follow-up dose response experiments. As these markers are important indicators of cell growth and death, understanding how specific chemical moieties dictate their expression is crucial to developing a therapeutic with the precise, intended impact on cancer cells. These results present evidence that rocaglates have contrasting impacts on the same leukemia cells and lay the groundwork for further investigation into the structure-bioactivity relationships within this promising class of molecules. While this proposal focuses on rocaglates as a specific test case, this pipeline will be broadly applicable for elucidating structure-function relationships for other promising families of therapeutics.

C11. Structure of yeast ALA synthase reveals divergent mechanisms of enzyme autoregulation governing heme biosynthesis

Jenny U. Tran, Breann L. Brown

The first and rate-limiting step of heme biosynthesis is the production of aminolevulinic acid (ALA). This reaction is catalyzed by ALA synthase (ALAS). ALAS contains a highly conserved core, but eukaryotic homologs have evolved to include divergent C-terminal extensions (Ct-ext). Yeast The ALAS Ct-ext has been shown to have differential autoregulatory roles. In humans, the Ct-ext has an autoinhibitory function. In yeast, truncation leads to a decrease in activity.

D. THERAPEUTICS AND TRANSLATION

D1. Overexpression of Alveolar Epithelial Tissue Factor Promotes Maintenance of Lung Barrier Integrity in ALI

Brandon Baer, Nathan D. Putz, Noo Ri Lee Han, Lorraine B. Ware, Julie A. Bastarache

Acute Respiratory Distress Syndrome (ARDS) is a common cause of acute respiratory failure. Despite extensive research in animal models, in which the syndrome is called Acute Lung Injury (ALI), no targeted therapy has been found to reduce its high mortality rate. Two major pathologic features of ARDS are loss of lung barrier integrity and activation of the Tissue Factor (TF) pathway of coagulation in the airspace. However, as an integral membrane protein TF also serves several non-coagulant functions including promotion of cell adhesion. All systemic anti-coagulants tested have failed to show clinical benefits in ARDS, with some trials of TF pathway inhibition showing increased mortality in ARDS patients. One explanation for these clinical results is that **TF in the airspace is protective in ARDS**. Supporting this concept, our previously published mouse work found that loss of alveolar epithelial cell TF caused increased loss of lung barrier integrity in models of ALI. As such, we hypothesize that epithelial TF is necessary for maintaining lung barrier integrity and that its overexpression will be protective in ALI. To determine whether supraphysiologic overexpression of TF in the lung can enhance barrier integrity we created a novel transgenic mouse in which TF was inducibly overexpressed in the lung epithelium (TF^{Epi+}). Specifically, a human influenza hemagglutinin-tagged TF construct, driven by the CMV-TetO promoter and crossed with SPC-rtTA59 mice was used to produce inducible, lung epithelial-targeted TF overexpressing mice. High alveolar epithelial TF expression compared to wild-type littermates (WT) was confirmed through immunohistochemistry and western blot analysis after one week of doxycycline in drinking water. To induce ALI, mice were intranasally infected with 2000 colony forming units of Klebsiella pneumoniae or PBS. At 24-hours post infection, mice were euthanized, lung tissue was collected, and a bronchoalveolar lavage (BAL) was performed. Animal body weights were recorded pre-, and 24-hours post infection. BAL was analyzed to measure protein, clot time, and leukocyte influx. Lung tissue was utilized to calculate wet-to-dry weight ratios and bacterial burden. TF^{Epi+} mice infected with Klebsiella pneumonia showed lower BAL protein (385.63 vs 248.51 µg/ml; n=16; p=0.0019) and lung wet-to-dry weight ratios (5.24 vs 4.86; n=16; p=0.0560) compared to WT. However, weight loss, bacterial burden, BAL clot time, and BAL inflammatory cell counts did not differ between infected TF^{Epi+} and WT mice. These findings suggest that alveolar epithelial TF overexpression is protective for maintaining lung barrier integrity and a non-coagulate based mechanism, potentially linked to epithelial cell adhesion.

D2. *Clostridioides difficile* increases undecaprenyl pyrophosphate recycling and drug efflux in response to iron starvation.

Martin V. Douglass and Eric P. Skaar

nosocomial intestinal infection in the United States and an urgent threat to public health. CDI onset begins with C. difficile outcompeting both the host microbiota and the innate immune response for limited nutrients. A critical factor in the host immune response to CDI is the innate immune protein calprotectin (CP) that chelates essential nutrient metals from the pathogen through a process termed nutritional immunity. CP is essential for the host to combat CDI, yet how C. difficile overcomes CP to acquire nutrients is not well understood. To uncover how C. difficile responds to nutritional immunity, we evaluated the transcriptional changes that C. difficile undergoes when challenged with CP. We identified a putative two-component system (TCS), 2822 and 2823, to be transcriptionally increased in the presence of CP and iron chelators. Mutants lacking this TCS exhibit a growth defect in iron limiting conditions. Furthermore, we found 2822/2823 regulates three genes immediately downstream: 2821, 2820, and 2819. Based on bioinformatic predictions, 2820 and 2819 encode an ATP driven efflux pump, and 2821 encodes an undecaprenyl pyrophosphatase. Further experiments revealed that 2822/2823 is activated by the antibiotic bacitracin, and mutants lacking the TCS are extremely sensitive to the cell surface targeting molecules bacitracin and vancomycin, the latter of which is clinically relevant. Our results support a model in which C. difficile overcomes nutritional immunity by coordinating an increase in undecaprenyl pyrophosphate recycling and drug efflux to defend against external threats such as iron restriction and cell envelope targeting antimicrobials.

D3. Drug repurposing: evacetrapib as a candidate to treat long QT syndrome type 2.

Christian Egly, Dave Weaver, Björn C Knollmann

The potassium channel $K_V11.1$ plays an important role in repolarization of cardiac action potentials and loss-of-function (LOF) $K_V11.1$ variants cause Long QT Syndrome, which predisposes individuals to fatal arrhythmias. Approximately 90% of LOF variants prevent $K_V11.1$ intracellular transport (trafficking) to the plasma membrane and prolonged incubation with drugs can improve $K_V11.1$ trafficking and increase current generated by the channel after washout. **Objective:** Develop a high-throughput screen to identify clinically approved drugs that increase $K_V11.1$ trafficking and function. **Methods:** Using an optimized Tl^+-flux assay, we screened 1906 drugs using HEK-293 cells expressing $K_V11.1$ trafficking-deficient variants ($K_V11.1$-G601S-G965*X and $K_V11.1$-N470D) for increased $K_V11.1$ trafficking. Cells were plated on 384-well, clear bottomed plates with 10 µM drug in individual wells 24-hours before experiments. On the day of experiments, drug was washed out, loaded with thallium-sensitive dye, and imaged using a 384-well fluorescent plate reader. Drugs with B-scores ≥3 (see analysis) were considered positive hits from the screen. **Results:** The screen detected hundreds of drugs that increased $K_V11.1$ variant trafficking. Unfortunately, most hits inhibit $K_V11.1$ acutely, so we screened acute and overnight drug effects on $K_V11.1$-WT and eliminated drugs that decrease $K_V11.1$-WT function. Evacetrapib not only increased $K_V11.1$ variant trafficking, but activated currents suggesting a novel, dual mechanism. **Conclusion:** We discovered the clinical drug

evacetrapib increased trafficking and function of multiple K_V11.1-variants at concentrations comparable to human plasma levels and is an ideal candidate to treat LQTS.

D4. CFTR Interactome remodeling by Elexacaftor (VX-445) reveals differential correction driven by translational dynamics.

Minsoo Kim, Eli F McDonald, Carleen Mae P Sabusap, Bibek Timalsina, Lars Plate

Cystic Fibrosis is caused by a loss-of-function mutation in CFTR. The proteostasis network is reshaped by CFTR mutations and corrector drugs. Effective correctors restore mutant interaction profiles back to WT. Aberrant P67L shows increased interactions with translation factors. siRNA KD of ribosomal proteins (RPL 34 and RPL 37) sensitized P67L to VX-445. Our multiplexed approach can facilitate characterization of altered proteostasis interactions of theratypes for future CFTR correctors, other protein misfolding diseases. Future work to assess translation speed, polysome composition, and ribosomal footprinting in response to RPL KDs will be required to further explain the sensitization.

D5. An AT3 family acyltransferase participates in *Acinetobacter baumannii* nutrient metal acquisition and virulence

Dillon E. Kunkle, Matt J. Munneke, & Eric P. Skaar

Acinetobacter baumannii is a multidrug-resistant nosocomial bacterial pathogen that causes a range of diseases including respiratory and wound infections. *A. baumannii* is the leading cause of hospital-acquired pneumonia and has been identified as a major pathogen coinfecting COVID19 patients. The WHO has categorized *A. baumannii* as the most critical bacterial pathogen for the development of new therapeutics. Nutrient transition metals are essential to all life forms, including pathogenic bacteria. Vertebrates exploit this requirement by sequestering metals from invading pathogens in a process known as nutritional immunity. We have shown that the struggle for nutrient metals at the host-pathogen interface is a critical determinate of *A. baumannii* infection outcome. However, the mechanisms that *A. baumannii* employs to respond to, and overcome, nutritional immunity remain poorly understood. We have identified a gene, *A1S_3410*, which encodes for a membrane bound AT3 family acyltransferase that is induced during nutrient metal limitation. Our findings indicate that A1S_3410 targets cytoplasmic proteins for acyl-transfer, in contrast to AT3 acetyltransferase homologues which target extracellular carbohydrates. Loss of *A1S_3410* results in a reduced capacity to survive in metal-limiting environments, diminished metal acquisition, and attenuated virulence in a mouse model of pneumonia. Collectively, these results suggest a previously unappreciated role of post-translational protein modification in the maintenance of bacterial metal homeostasis.

D6. Targeting the Peripheral Myelin Protein 22 Using Fragment Based Drug Discovery.

Geoffrey Li, Thilini Ukwaththage, Charles R. Sanders

Charcot-Marie-Tooth Disease (CMT) is a hereditary disorder of the peripheral nervous system that affects 1 in 2500 humans with no available treatment yet. CMT is characterized by a progressive loss of function and sensation in hands, arms, legs, and feet. Over 75% of cases are due to genetic variation in PMP22 gene encoding for peripheral myelin protein 22 (PMP22). PMP22 is a 160-residue tetraspan membrane protein. PMP22 comprises 2-5% of total protein in compact myelin. Misfolding and aggregation of PMP22 is believed to cause CMT. This project aims to find small molecules that bind to PMP22 that could act as folding correctors or improve the cell surface expression of PMP22.

D7. A Sweet Treat: Employment of human milk oligosaccharides as adjuvants in the antimicrobial arms race

Sabrina K. Spicer, Jennifer A. Gaddy, Steven D. Townsend

Human breast milk has long been coveted as the gold standard for infant nutrition. Containing a heterogenous mixture of fats, proteins, and other necessary nutrients, breast milk supplies complete nutrition to the neonate up to 2 years of life. Among the bioactive molecules found in human breast milk are human milk oligosaccharides (HMOs). While not digestible by the infant, these sugar molecules are widely known to promote the growth of commensal bacteria in the infant gut while suppressing the growth of pathogenic bacteria. We have previously characterized the impressive antimicrobial and antibiofilm activities of these molecules with gram positive pathogens at length. Interestingly, we have recently found that while the robust antibiofilm phenotype persists HMOs possess no antimicrobial power over *Acinetobacter baumanni*, an urgently threatening gram-negative pathogen. Biofilm-inhibiting compounds have recently gained attention as a potential chemotherapeutic strategy to prevent or dismantle *A. baumannii* biofilms and restore the utility of antimicrobial strategies. Recent work indicates that human milk oligosaccharides (HMOs) have potent antibacterial and biofilm-inhibiting properties against a bank of *A. baumannii* clinical isolates. With these data in hand, we sought to test the utility of HMOs as antibiotic adjuvants against the multidrug resistant isolates of *A. baumannii* to underscore the impact of biofilm formation in the antimicrobial resistance crisis. Our results indicate that the potent anti-biofilm activity of HMOs confounds antimicrobial resistance mechanisms within this gram-negative pathogen to revive otherwise ineffective antibiotics, including various carbapenems, a last line of defense antibiotic class for these types of infections. We report here the impressive utility of this therapeutic activity, a proteomic analysis revealing the acetoin pathway as an important determinant in HMO-microbe-host interactions, and other discovery strategies used to underscore the molecular underpinnings governing HMO-bacterial interactions. Preliminarily speaking, HMOs could be the light at the end of an exhausting tunnel of antimicrobial resistance.

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