2021
August 12th

VANDERBILT INSTITUTE OF CHEMICAL BIOLOGY
VIRTUAL STUDENT RESEARCH SYMPOSIUM

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* (Casey Butrico, Chair)	
8:35 am – 8:55 am	**Rebecca Moore (Townsend Research Group)** *Leveraging human milk oligosaccharidesat the host-pathogen interface*
8:55 am – 9:00 am	**Break**
9:00 am – 9:20 am	**Lianyan Xu (Townsend Research Group)** *Bioorganic Investigation of Desgalactotigonin*
9:20 am – 9:25 am	**Break**
9:25 am – 9:50 am	**John Wilson (Associate Professor of Chemical, Biomolecular, and Biomedical Engineering)** *Molecularly Engineered Technologies for Immuno-Oncology*
9:50 am -10:00 am	**Break**
Oral Session II *Molecular Discovery and Systems Analysis* (Madison Wright, Chair)	
10:00am-10:20 am	**Ricardo Capone (Sanders Research Group)** *Recombinant SARS-CoV-2 envelope protein traffics to the trans-Golgi network following amphipol-mediated delivery into human cells*
10:20am-10:25 am	**Break**
10:25am- 10:45 am	**Kavya Sharman (Caprioli Research Group)** *Automated Analysis of Highly Dimensional Spatially Targeted Proteomics Data*
10:45am- 10:50am	**Break**
10:50am-11:15 am	**Ray Blind (Assistant Professor of Medicine, Pharmacology, and Biochemistry)** *New Mechanisms of Nuclear Receptor Activation Inform Compound-Screening Strategies*
11:15am-11:20 am	**Break**
11:20am-11:35am	VICB Citation Winner Flash Talks: **Andrea Pruijssers, Ph.D. (Denison Research Group)** *In Search of a Magic Bullet: Development of Orally Bioavailable Coronavirus Antivirals*
11:35am – 12:05pm	**Christine Brideau (Vice President of In Vitro Pharmacology, Deerfield Discovery and Development)** *Translating ideas into therapeutics in partnership with 3DC*
LUNCH BREAK 12:05 pm – 1:00 pm	
12:30 pm – 1:00 pm	**Q & A with Dr. Schreiber (RSVP-only event)**
AFTERNOON SESSION	
Oral Session III *Richard Armstrong Prize for Research Excellence* (Henry Schares, Chair)	
1:00 pm - 1:30 pm	**Benjamin Reisman, Prize Runner Up (Bachmann Research Group)** *A Family of Glycosylated Macrolides Selectively Target Energetic Vulnerabilities in Leukemia*
1:30 pm - 2:00 pm	**Madison Wright, Prize Winner (Plate Research Group)** *Protein-Protein Interactions in Quality Control: From Steady-State Interactions to Time-Resolved Dynamics*
2:00 pm - 2:10 pm	**Break**

Oral Session IV *Keynote* (Amanda Cao, Chair)	
2:10 pm - 3:10 pm	**Dr. Stuart Schreiber (Morris Loeb Professor of Chemistry and Chemical Biology, Harvard University, Howard Hughes Medical Institute Investigator, & Core Member of the Broad Institute)** *30 years of Molecular Glues: Controlling cell circuitry in biology and medicine*
3:10 pm – 3:15 pm	**Break**
3:15 pm – 4:00 pm	**Academic and Industry Relations Panel** **Stuart Schreiber, Ph.D.,** Morris Loeb Professor of Chemistry and Chemical Biology, Harvard University, Howard Hughes Medical Institute Investigator, & Core Member of the Broad Institute **Christine Brideau,** Vice President of In Vitro Pharmacology, Deerfield Discovery and Development **Aleksandra Baranczak, Ph.D.,** Alliance Manager at The Scripps Institute **Moderated by George Wilson,** New Ventures Officer, Vanderbilt Center for Technology Transfer and Commercialization (CTTC)
RECEPTION	
4:00 pm – 5:00 pm	**Poster session on Gather Town** https://gather.town/app/H4IkofC1SiZCHRLE/VICB%20Poster%20Session

"30 years of Molecular Glues: Controlling cell circuitry in biology and medicine"

Stuart L. Schreiber Ph.D.

Morris Loeb Professor of Chemistry and Chemical Biology at Harvard University, a co-Founder of the Broad Institute, and a member of the National Academy of Sciences, National Academy of Medicine and American Academy of Arts and Sciences

Dr. Schreiber's research integrates chemical biology and human biology to advance both our understanding of chemistry and biology, and the discovery of novel therapeutics. He is known for his use of small molecules to explore biology and medicine, and for his role in the development of the field of chemical biology. With Jerry Crabtree in 1991, his lab mapped the first membrane to nucleus signaling pathway (calcium–calcineurin–NFAT). His lab co-discovered mTOR in 1994 (simultaneously with Sabatini) and helped illuminate the mTOR-dependent nutrient-response signaling network. His lab discovered histone deacetylase (HDAC) and, together with David Allis and Michael Grunstein in 1996, the role of chromatin marks in gene expression. His work demonstrated for the first time that drugs can result from: 1) the targeting of protein kinases (sirolimus/mTOR) and protein phosphatases (sandimmune/ calcineurin); 2) gene regulation by chromatin-modifying enzymes (vorinostat/HDAC), 3) chemical inducers of proximity (CIPs) that activate cellular processes by enforced proximity (GVH Disease), and 4) targeting of the proteasome (bortezomib/proteasome). His research illustrated that many small molecules are bifunctional and act by inducing proximity of signaling proteins, which he coined 'molecular glues'. The discovery of molecular glues and development of chemical inducers of proximity led conceptually to the targeted degradation of proteins by small-molecule 'PROTACs'. These efforts accelerated the development of many additional widely used drugs and more generally the field of chemical biology.

Schreiber's development of diversity-oriented synthesis has led to the discovery of many promising agents, including a novel mechanism of action anti-malarial agent being developed in collaboration with the pharmaceutical company Eisai (*Nature*, 2017). His most recent discovery revealed a novel cell state responsible for the ability of cancers to resist a wide range of therapies, and a means to target the cancer therapy-resistant state (*Nature*, 2017). His research has been reported in over 650 publications (H index = 147; Schreiber Publications) and recognized through numerous awards, most recently the Arthur C. Cope Award and the Wolf Prize in Chemistry.

Four new anti-cancer drugs that target proteins discovered in the Schreiber laboratory have been approved by the U.S. FDA: temsirolimus (Wyeth) and everolimus (Novartis), which target mTOR (discovered using rapamycin in 1994), for renal cancer, and vorinostat (Merck) and romidepsin (Celgene), which target HDACs (HDAC1 discovered using trapoxin in 1996), for cutaneous T-cell lymphoma; in addition, topical HDAC inhibitor remetinostat (Medivir, PII clinical trials for CTCL), was conceived and synthesized in the Schreiber lab. A small-molecule molecular-glue drug (AP1903) reversed the effects of graft-versus-host disease in acute leukemia patients receiving hematopoietic stem cells engineered to express caspase-9 fused to a drug-responsive, FKBP12-based dimerization domain (NEJM, 2011). Proteins first shown by Schreiber to be targeted by a small molecule have been validated therapeutically by the FDA-approval process: tacrolimus (calcineurin/immunosuppression/1994; Schreiber's study of FK506) and bortezomib (proteasome/multiple myeloma/2003; Schreiber's study of lactacystin).

Schreiber extended chemical biology principles to medicine by participating in the founding of ten biotech companies, beginning with Vertex Pharmaceuticals, whose efforts have made cystic fibrosis a manageable disease. These companies have developed many novel therapeutic agents that are being tested in human clinical trials or used as FDA-approved drugs including: Vertex Pharmaceuticals (founded 1989: fosamprenavir/Lexiva; telaprevir/Incivek; ivacaftor/Kalydeco), ARIAD Pharmaceuticals (founded 1991: ponatinib/Iclusig; brigatinib/Alunbrig), ARIAD Gene Therapeutics (founded 1994: ridaforolimus; AP1903), and Infinity Pharmaceuticals (founded 2001: retaspimycin; duvelisib). Earlier stage chemical biology-based companies formed by Schreiber include: Forma Therapeutics, H3 Biomedicines, Jnana Therapeutics, Kisbee Therapeutics, Kojin Therapeutics and oNeir Therapeutics. In 2020, Schreiber co-founded Scientists to Stop COVID-19, a nonpartisan science-based group who advise policy makers in U.S. executive, congressional and state governments, as well as leaders in the sports and entertainment industries.

In Search of a Magic Bullet: Development of Orally Bioavailable Coronavirus Antivirals

Andrea J. Pruijssers, Ph.D. is a Research Associate Professor of Pediatrics at Vanderbilt University Medical Center (VUMC). She received a M.S. degree in Cell and Molecular Biology from Wageningen University in The Netherlands and a Ph.D. in Entomology from the University of Georgia. Dr. Pruijssers directs the Coronavirus Antivirals Program in the laboratory of Mark Denison, M.D. The Denison lab is one of the few laboratories in the world that has researched Coronaviruses for decades. Dr. Pruijssers has studied a variety of neat viruses over the past two decades. She joined Dr. Denison's laboratory just in time to get up to speed with MERS-CoV before the worldwide SARS-CoV-2 pandemic started. Dr. Pruijssers and her coworkers were the first to quantify the neutralizing antibody response elicited by the Moderna mRNA vaccine in humans. However, effective small molecule antivirals for the treatment of coronavirus infections are still lacking. By targeting conserved viral proteins, the Program aims to develop broad-spectrum antivirals for the treatment of the current and future coronavirus pandemics. Her team's efforts in collaboration with industry partners has led to the development of Remdesivir and the orally bioavailable drug Molnupiravir for the treatment of COVID-19. Current research focuses on the development of oral drugs that target different stages of the viral replication cycle and that can be combined to increase drug activity spectrum and reduce resistance development.

Protein-Protein Interactions in Quality Control: From Steady-State Interactions to Time-Resolved Dynamics

Madison T. Wright, Lars Plate

Madison Wright, Winner

Cells are under pressure to properly fold, assemble, and maintain the integrity of thousands of proteins throughout changes in cellular conditions and stress. The proteostasis network (PN), consisting of folding, trafficking, and degradation components, helps facilitate the proper processing, trafficking, localization, and secretion of these proteins in a highly coordinated process referred to as protein quality control (PQC). Protein misfolding diseases such as thyroglobulin related congenital hypothyroidism (CH) are a result of improper partitioning of proteins between PQC pathways. We have utilized a quantitative affinity purification – mass spectrometry (AP-MS) method and revealed that CH-associated thyroglobulin mutations exhibit PN-interaction imbalances within PQC pathways associated with post-translational modifications such as glycosylation & disulfide bond formation, protein folding, and degradation. Coordination of PN-client interactions are critical for mediating proper PQC decisions, yet the timing of these interactions are only understood for a few PN components. One limitation is the lack of methodologies available to identify and quantify transient protein-protein interactions with time-resolution on an organelle wide level. We are therefore developing a quantitative mass spectrometry method, time-resolved interactome profiling (TRIP), to characterize the coordination and sequential interactions between client proteins and the PN to better understand PQC processes. TRIP utilizes unnatural amino acid incorporation and streptavidin-biotin enrichment to label and subsequently purify nascent proteins into time-resolved fractions for AP-MS analysis. Our goal is to temporally resolve common PQC processes that have remained incompletely characterized, and further identify key changes in protein-protein interactions that take place from WT to CH-associated thyroglobulin mutations. Ultimately, this may reveal mechanisms available for therapeutic targeting, and provide insight into the PN and its PQC processes on the whole that have broader applicability in other protein misfolding diseases.

A Family of Glycosylated Macrolides Selectively Target Energetic Vulnerabilities in Leukemia

Benjamin Reisman, Brian O. Bachmann

Benjamin Reisman, Runner up

The development of effective anti-cancer therapeutics is constrained by the need to identify targeted and drugs that selectively eliminate cancer cells with limited toxicity towards healthy cells. Though alterations in cellular bioenergetics have long been recognized as a hallmark of cancer, recent studies have demonstrated that leukemic cells, particularly acute myeloid leukemia cells, are uniquely dependent on oxidative phosphorylation (OXPHOS) to serve their bioenergetic and biosynthetic needs. The apoptolidin family of glycosylated macrolides (glycomacrolides) was discovered in a series of phenotypic screens for compounds that induce apoptosis selectively in oncogene-transformed cells. While studies on the mode of action of this class suggested they acted as inhibitors of OXPHOS, their molecular target and mechanism of action has remained unconfirmed. To resolve this question, we used photoaffinity analogs of the apoptolidins to identify the F_1 subcomplex of mitochondrial ATP synthase as the target of apoptolidin A. CryoEM of apoptolidin and ammocidin-ATP synthase complexes revealed a novel shared mode of inhibition that was confirmed by deep mutational scanning of the binding interface to uncover resistance mutations which were confirmed using CRISPR-Cas9 genome editing. Ammocidin A was found to suppress leukemia progression in vivo at doses that were tolerated with minimal toxicity. This combination of cellular, structural, mutagenesis, and in vivo evidence define the mechanism of action of apoptolidin family glycomacrolides and establish a path to address OXPHOS-dependent cancers.

Stuart Schreiber, Ph.D., Morris Loeb Professor of Chemistry and Chemical Biology at Harvard University, a co-Founder of the Broad Institute, and a member of the National Academy of Sciences, National Academy of Medicine and American Academy of Arts and Sciences

Christine Brideau, Vice President of In Vitro Pharmacology, Deerfield Discovery and Development

Christine Brideau joined Deerfield in 2019 as Vice President of In Vitro Pharmacology for Deerfield Discovery and Development, LLC. Christine joined Deerfield from WuXi AppTec, where she was Vice President of In Vitro Pharmacology and led the development of the US-based in vitro biology unit in New Jersey. Prior to WuXi, she spent 24 years at Merck, where she most recently served as Executive Director of In Vitro Pharmacology and supported multiple drug discovery projects from hit-to-lead to clinical candidate. She is the co-author of more than 40 peer-reviewed publications in the areas of pharmacology, assay development, screening data analysis, automation and compound management. Christine is currently a member of the Scientific Advisory Board of CQDM (Quebec Consortium for Drug Discovery) and served 6 years as a member of the editorial board member of SLAS Discovery and for the NIH Molecular Libraries Program study sections.

Aleksandra Baranczak, Ph.D.,

Aleksandra Baranczak is an Alliance Manager at The Scripps Research Institute and its drug discovery division - Calibr. In her role, she is responsible for establishment and management of collaborations with academic and industry partners. Prior to joining Scripps, she led a chemical biology group at the pharmaceutical company AbbVie and worked in the area of research development at the University of Chicago. Aleks received her PhD in Chemistry from Vanderbilt University followed by postdoctoral studies in chemical biology at Scripps Research.

Moderator: George Wilson, New Ventures Officer, Vanderbilt Center for Technology Transfer and Commercialization

George is a seasoned biomedical researcher with a broad scope of interest, including pharmacology, biochemistry, oncology, electrophysiology, and medical imaging. He holds a B.A. in Clinical Psychology and Medicine, Health, & Society, as well as a M.S. in Neuropharmacology and Imaging Methods, from Vanderbilt University's College of Arts & Science and School of Medicine, respectively. He is also a certified Project Management Professional. His role evolved from researcher to manager as he progressed through his research career, culminating in his directing a portfolio of industry-sponsored research projects as chief operations officer of an ophthalmology biotech start-up. George has further broadened his scope of interest in his present role as New Ventures Officer at Vanderbilt University's Center for Technology Transfer and Commercialization (CTTC), which is tasked with optimizing the flow of innovation from the lab to the marketplace. Vanderbilt's impressive research funding translates to commercialization opportunities for a wide variety of technologies. More specifically, George is responsible for supporting start-ups and helping to build out the entrepreneurial ecosystems at Vanderbilt and in Nashville.

CHEMICAL SYNTHESIS

Bioorganic Investigation of Desgalactotigonin

Lianyan L. Xu and Steven D. Townsend

Anthracyclines are clinical chemotherapeutics that eradicate cancer cells by inhibiting the ability of topoisomerase to relax supercoiled DNA. Interestingly, Desgalactotigonin (DGT) targets topoisomerases, even though it does not share structural homology with anthracyclines. We hypothesize that DGT, a steroidal glycoside, can be used to eradicate anthracycline resistant cell lines. Moreover, if this hypothesis is correct, it is likely that the cytotoxicity will not be accompanied by cardiotoxicity, as DGT lacks the anthracycline central quinoline aglycone that can generate reactive oxygen species (ROS). To test the central hypothesis, we will complete a total synthesis of Desgalactotigonin using a convergent glycosylation strategy, which harnesses anchimeric assistance from a C2-ester to promote β selectivity during each glycosylation event. A highlight of the synthesis includes installation of a challenging glycosidic bond formation between a glucoside donor and a weakly nucleophilic axial C4-alcohol of a galactose acceptor. To discover a minimal pharmacophore that can be used as a tool compound to further elucidate the mechanism of action several truncated congeners will be evaluated.

MOLECULAR DISCOVERY

Recombinant SARS-CoV-2 envelope protein traffics to the trans-Golgi network following amphipol-mediated delivery into human cells

James M. Hutchison, Ricardo Capone, Dustin D. Luu, Karan H. Shah, Arina Hadziselimovic, Wade D. Van Horn, and Charles R. Sanders

The SARS-CoV-2 envelope protein (S2-E) is a conserved membrane protein that is important for coronavirus assembly and budding. Here, we describe the recombinant expression and purification of S2-E in amphipol-class amphipathic polymer solutions, which solubilize and stabilize membrane proteins, but do not disrupt membranes. We found that amphipol delivery of S2-E to pre-formed planar bilayers results in spontaneous membrane integration and formation of viroporin cation channels. Amphipol delivery of the S2-E protein to human cells results in plasma membrane integration, followed by retrograde trafficking to the trans-Golgi network (TGN) and accumulation in swollen perinuclear LAMP1-positive vesicles, likely lysosomes. Coronavirus envelope proteins have previously been proposed to manipulate the luminal pH of the TGN, which serves as an accumulation station for progeny coronavirus particles prior to cellular egress via lysosomes. Delivery of S2-E to cells will enable chemical biological approaches for future studies of SARS-CoV-2 pathogenesis and possibly even development of “Trojan Horse” anti-viral therapies. Finally, this work also establishes a paradigm for amphipol-mediated delivery of membrane proteins to cells.

THERAPEUTICS AND TRANSLATION

Leveraging human milk oligosaccharides at the host-pathogen interface

Rebecca E. Moore, Jennifer A. Gaddy, and Steven D. Townsend

One of the most challenging problems facing the United States healthcare establishment is combatting hospital-acquired infections caused by bacteria that have developed antibiotic resistance. Several species of bacteria are responsible for this serious threat, including the Gram-positive, opportunistic bacterium Streptococcus agalactiae (Group B Streptococcus, GBS). Many strategies are currently employed in the clinic to prevent and treat GBS infections, one of which is combination therapy in which an adjuvant is administered along with the drug as this not only helps increase its efficacy, but also helps with the antibiotic resistance problem by lowering the dosages of the drugs required. We are interested in exploring the putative antimicrobial activity conferred by human milk oligosaccharides (HMOs) and its ability to act as an adjuvant. In previous work, we have demonstrated that HMOs can inhibit both growth and biofilm formation against GBS, as well as increase the efficacy of intracellular-targeting antibiotics through increased cell membrane permeability. We have also shown that GBS adheres to and forms biofilms on gestational membranes (the membranes that surround a fetus), and HMOs are able to disrupt both adherence and biofilms. Using these results, we hypothesized that we could prevent ascending infection of GBS during pregnancy and confirmed this in our mouse model. These results could have immense implications on adverse pregnancy outcomes including preterm birth, intrauterine infection, and neonatal sepsis and pneumonia.

SYSTEMS ANALYSIS

Automated Analysis of Highly Dimensional Spatially Targeted Proteomics Data

Kavya Sharman, Nathan Heath Patterson, Elizabeth K. Neumann, Andy Weiss, Emma R. Guiberson, Danielle B. Gutierrez, Raf Van de Plas, Eric P. Skaar, Richard M. Caprioli, Jeffrey M. Spraggins

Spatially targeted proteomics is a recent, powerful experimental approach for analyzing the proteome of tissue substructures. Nevertheless, interpreting the complex protein profiles of key regions with a substantial number of missing values presents computational challenges. Here, we present a multivariate proteomics data analysis workflow that provides biological insight from sparse spatially targeted proteomics data 4- and 10-days post-infection in a Staphylococcus aureus-infected murine kidney. The workflow is a multivariate data analysis pipeline employing principal component analysis (PCA) for dimensionality reduction and grouping of correlated and anticorrelated protein groups among regions and timepoints collected by microLESA. We then applied k-means clustering on the PCA-processed data, assigning samples membership in an unsupervised manner based on the underlying proteome. Finally, we interpreted the clustered centers using network analysis to uncover functional enrichments pertinent to infection, such as fatty acid oxidation, beta-oxidation, purine nucleoside detoxification, and cytoskeletal reorganization. Through this analysis, we observed differential proteomic changes across abscess regions over time, highlighting the dynamic nature of abscess formation. This work demonstrates that our approach overcomes the challenge of analyzing sparse spatially targeted proteomics data and provides a new understanding of the complex biology during staphylococcal infection progression.

A. CHEMICAL SYNTHESIS

A1. Design, Development, and Validation of Probe Molecules to Study Time-Resolved Interactomics

Crissey Cameron, Robert Mason Clark, and Lars Plate

Understanding protein interaction networks can give insight into the inner workings of systems biology, linking seemingly unrelated pathways to one another and deepening our understanding of the cellular processes controlled by protein interactions. Many approaches have been used in the literature to tease out static interactions of important proteins, but most fail to account for the dynamics and transient nature of many protein complexes and fail to map interactions with time resolution. Because of this, only a snapshot of the protein interactions at one point in time are accessible, painting an incomplete picture of the interactome. Chemical genetic probe molecules designed specifically to rescue a protein of interest from degradation by the proteosome allow for controlled accumulation of proteins in the cell, therefore allowing time-resolved study of a protein of interest. My research has designed a system in which a protein of interest is linked to a mutant dihydrofolate reductase (dDHFR) domain that is marked for degradation by the proteosome. This dDHFR can be rescued from degradation and allowed to accumulate in the cell using the small molecule trimethoprim. A cysteine mutation has been made near the active site in position L28, which allows for nucleophilic attack and covalent linking to nearby electrophiles. A probe molecule made up of a trimethoprim moiety, a terminal alkyne Click-chemistry handle, and an electrophile allows for rescue of the protein of interest, bioorthogonal derivatization of the probe using a fluorophore or biotin for visualization or isolation, and covalent linking of the protein of interest and the probe. This system has been validated using yellow fluorescent protein (YFP) as a model, showing time-dependent accumulation of YFP and the ability to isolate YFP from cell lysates. Further validation of this system is currently being conducted to study the time-resolved interactome of the proteins KRas and coronavirus nonstructural proteins. Mutations in KRas are associated with colorectal cancer and nonstructural proteins of coronaviruses SARS1, SARS2, and MHV are involved in organelle remodeling or immune suppression, making their interactomes relevant for investigation. By combining quantitative proteomics using TMT-tags with this time-resolved system, the sequential protein interactions can be studied.

A2. Enhanced Delivery of Drug Cargo via Endosomolytic Polymersomes

Payton T. Stone, Hayden M. Pagendarm, John T. Wilson

With current cancer treatments such as chemotherapy, radiation, and surgical procedures offering aggressive forms of treatment with little therapeutic efficacy, cancer immunotherapy demonstrates the potential to improve upon these limitations. Particularly, the delivery of RNA therapeutics to the tumor site can increase the expression of immunostimulatory genes leading to a downstream signaling cascade, ultimately resulting in an increase in the production of proinflammatory cytokines and antiviral interferons resulting in a more potent antitumoral immune response. However, these molecules are often rapidly cleared in vivo before they can reach the tumor site and have displayed limited efficacy as a result. In addition, these therapeutics must bypass the cell membrane and enter the cytosol to activate these intracellular signaling pathways. Herein, we detail the fabrication and characterization of endosomolytic polymeric nanoparticles (polymersomes) capable of encapsulating and protecting anticancer RNA therapeutics for improved delivery to the tumor site. Specifically, pH-responsive poly(ethylene glycol)-block-[2-diethylamino)ethyl methacrylate-co-butyl methacrylate] (PEG-bl-[DEAEMA-co-BMA]) copolymers were synthesized via reversible addition-fragmentation chain-transfer (RAFT) polymerization. One of the main goals of this work was to examine the effect of copolymer properties on nanoparticle structure and function. Specifically, we varied the second block molecular weight from 6 kDa to 20 kDa while keeping the first block molecular weight constant at 2 kDa. Nanoparticles were formulated via a facile and highly scalable flash nanoprecipitation (FNP) process in which an organic inlet stream containing the copolymer was impinged against an aqueous phase containing the hydrophilic drug. The turbulent mixing of the two streams in a confined impingement jet (CIJ) mixer allowed for the self-assembly of nanoparticles and simultaneous encapsulation of drug cargo. It was found that increasing copolymer second block molecular weight resulted in an increase in nanocarrier size and polydispersity. Further, nanocarriers were found to degrade and lyse erythrocytes in vitro upon a decrease in pH consistent with endosomal acidification. In addition, it was found that samples with a second block molecular weight of ≤ 12 kDa remained stable when stored at 4℃ over four weeks, and nanocarriers were able to encapsulate a variety of hydrophilic cargo, including a 25-mer NC-DNA duplex. In this work, we highlight a means of production of nanoparticles of varying structure and properties. Such a system displays the potential to be implemented into a large-scale process centered on efficiently encapsulating RNA therapeutics within polymeric nanoparticles for improved delivery in vivo.

A3. Structural Effects of incorporation 6-oxo-M1dG DNA adduct into DNA Duplex

Yizhi Fu

3-(2-Deoxy-ß-D-*erythro*-pentofuranosyl)pyrimido[1,2-ð]purin-10(3*H*)-one (M_1dG) is an endogenously exocyclic lesion produced by DNA peroxidation products, base propenals, and it is repaired by nucleotide excision repair (NER). In both humans and rats, M_1dG is oxidized by xanthine oxidase and forms 6-oxo-M_1dG. Previous data showed the synchrony of the reduction of M_1dG and increasing of 6-oxo-M_1dG, this indicated after repaired by NER, free M_1dGs are oxidized to 6-oxo-M_1dG endogenously. Because M_1dG can be oxidized and 6-oxo-M_1dG is the sole metabolite, 6-oxo-M_1dG might be a better biomarker than M_1dG. Here, we present melting temperature data and NMR data which gave insight into structural effects of 6-oxo-M_1dG incorporation into DNA duplex. 6-oxo-M_1dG was constructed in the oligodeoxynucleotide 5'-d($C^1A^2T^3X^4A^5T^6G^7A^8C^9G^{10}C^{11}T^{12}$)-3':5'd($A^{13}G^{14}C^{15}G^{16}T^{17}C^{18}A^{19}T^{20}C^{21}A^{22}T^{23}G^{24}$)-3' (X=6-oxo-$M_1dG$). Melting temperature data showing the incorporation of 6-oxo-M1dG reduces the thermal stability of DNA duplex. NMR spectroscopies gave information to reveal an ordered structure for the 6-oxo-M_1dG DNA duplex and afforded detailed spectroscopic resonance assignments. According to the 2D NOESY spectrum, 6-oxo-M_1dG disrupts the DNA structure by disrupting the NOEs between 6-oxo-M_1dG and A^5, as well as the NOEs between T^{20} and C^{21}, base pairing neighbor of 6-oxo-M_1dG. 2D COSY and NOESY spectra both showing C^{21} is deshielded in the 6-oxo-M_1dG duplex, this indicated C^{21} might be pushed out of the helix. According to the 2D COSY, there may be a minor conformation of 6-oxo-M_1dG at low temperature. 6-oxo-M_1dG DNA duplex structure will be determined experimentally using molecular dynamics calculations restrained by NOE data. The minor conformation of 6-oxo-M_1dG will be further investigate by NMR.

A4. Discovery and Characterization of NAPE-PLD Activators

Jonah Zarrow, Kwangho Kim, Zahra Mashhadi, Isabelle C. Suero, Paige N. Vinson, Gary Sulikowski, and Sean S. Davies

Atherosclerosis is the primary cause of cardiovascular disease, which is a major health concern in the United States. It begins with lipid accumulation in cells in the arteries, which form foam cells that subsequently undergo apoptosis. Under normal conditions, these apoptotic cells are cleared by macrophages in a process called efferocytosis. However, during atherosclerosis, the efferocytotic capacity of macrophages appears to be overwhelmed, causing the apoptotic cells to undergo secondary necrosis. This leads to the formation of the necrotic cores that are typical of rupture-prone plaques, which ultimately lead to arterial blockage. Although cholesterol-lowering drugs are effective at reducing the initial atherosclerotic burden, there are currently no therapies that rescue efferocytosis or curtail necrosis in established lesions. Thus, there is a strong need for therapeutics targeting this pathway. Recent evidence suggests that the enzyme N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) may regulate efferocytosis. NAPE-PLD catalyzes the synthesis of N-acylethanolamides (NAEs), which exert potentially protective effects against cardiometabolic diseases. Importantly, certain NAEs, such as *PEA* and *OEA* have been shown to enhance macrophage efferocytosis. Additionally, NAPE-PLD expression is reduced in arteries during the development of atherosclerosis, suggesting that the loss of NAE biosynthesis plays a role in the progression of atherosclerosis. I hypothesize that pharmacologically activating NAPE-PLD will enhance macrophage efferocytosis. To test this hypothesis, I need NAPE-PLD activators; however, none have yet been identified. To solve that problem, I used a fluorescence-

based activity assay to screen 40,000 compounds, and identified 411 candidate activators. Subsequent studies identified a molecular scaffold that displayed sub-micromolar potencies in vitro. I will, therefore, characterize the potency, selectivity, and toxicity of these activators. I will then use them to study the role of NAPE-PLD in regulating efferocytosis by macrophages.

A5. Quantitative Proteomics to Study Aging in Rabbits

Bailey L. Bowser, Bushra Amin, Renã A. S. Robinson

Age is the biggest risk factor for Alzheimer's disease (AD) and age-related diseases such as diabetes and hypertension. While improvements in our understanding of the biology of aging have occurred due to advancements in 'omics technology, complete understanding of this process does not exist. To obtain additional perspective, rabbits can serve as an insightful model because of their 85% genetic homology to humans and longer lifespans than traditional rodent models (~8 years). We will follow proteomic changes in young, middle and old aged rabbits by utilizing a bottom up proteomics workflow in combination with combined precursor isobaric labeling and isobaric tagging (cPILOT) multiplexing strategy. cPILOT is a chemical labeling technique that allows for more than one biological sample to be injected on a mass spectrometer and enables studies of disease across multiple animal models and sample types. Herein, we will discuss the benefits of a 20-plex cPILOT approach and insights on aging in a rabbit model.

B. MOLECULAR DISCOVERY

B1. The catalytic mechanism of SRAP DNA-protein crosslinking

Katherine Amidon Paulin

Abasic (AP) sites are one of the most common DNA lesions, are prone to strand breaks, and can block DNA polymerases during replication. The SOS Response Associated Peptidase (SRAP) protein domain is conserved across all domains of life and forms a stable DNA-protein crosslink (DPC) to AP sites in single stranded DNA (ssDNA). This activity forms the basis of a novel DNA repair pathway whereby SRAP is proposed to protect AP sites from nucleases and error-prone polymerases. Structural and mutational analysis provides mechanistic roles of highly conserved active site amino acid residues in the DPC reaction, indicating a critical role for a conserved glutamate in DPC catalysis. Interestingly, the DPC reaction rate is pH-dependent, further supporting a catalytic role for the active site glutamate. Biochemical and structural evidence supports that the N-terminal amine is the initial crosslinking nucleophile and that the SRAP DPC reaction proceeds through a Schiff base intermediate. Further elucidating the role of SRAP in preserving genomic integrity, we demonstrate biochemically that SRAP can form DPCs with the 3' unsaturated aldehydes of cleaved DNA ends, consistent with a recently published crystal structure. Although the DPC is stable over time, we demonstrate that the DPC can be directly reversed, providing additional insight into possible pathways for SRAP-DPC resolution in cells.

B2. Resistance-Guided Mining of Bacterial Genotoxins Defines a Family of DNA Glycosylases

Noah P. Bradley, Katherine L. Wahl, Jacob L. Steenwyk, Lauren A. Washburn, Plamen P. Christov, Coran M. H. Watanabe, Gong-Li Tang, Antonis Rokas, Brandt F. Eichman

The bacterial enzymes AlkX and AlkZ are a recently discovered family of DNA glycosylases that repair interstrand DNA crosslinks formed from bifunctional DNA alkylating agents. Streptomyces AlkZ is found in the biosynthetic gene cluster of azinomycin B—a potent antimicrobial/antitumor agent—and provides self-resistance to the natural product, whereas Escherichia coli AlkX is located outside of a metabolic gene cluster, and excises a boarder range of substrates than AlkZ to provide crosslink resistance. The relationship between AlkX and AlkZ has not been extensively examined genetically, and the distribution of these families in biosynthetic gene clusters (BGCs) is unknown. Here, we analyze roughly 900 AlkX/AlkZ homologs from 435 Streptomyces species to determine their phylogenetic relationship and proximity to BGCs. Among the differences between these two subfamilies, AlkZ homologs are plastic in their genomic location and copy number, whereas AlkX is highly conserved sequence-wise and by genetic neighborhood and copy number. Using a resistance-based genome mining approach, 11 AlkZ homologs were identified in known BGCs, and 68 in uncharacterized BGCs; no AlkX homologs were in proximity to a cluster. We validated our method by characterizing HedH4 as an AlkZ homolog in the hedamycin BGC which uniquely excises hedamycin-DNA adducts and provides cross-resistance for E. coli. Our results support the role of AlkX/AlkZ-mediated base excision repair (BER) in antimicrobial resistance to a broad range of natural products, and allows for targeted discovery of DNA damaging BGCs through resistance genome mining (RGM).

B3. Identifying Requirements for RSK2 Specific Inhibitors

Eric B. Wright, Shinji Fukuda, Mingzong Li, Yu Li, George A. O'Doherty and Deborah A. Lannigan

Identifying isoform-specific inhibitors for closely related kinase family members remains a substantial challenge. The necessity for achieving this specificity is exemplified by the RSK family, downstream effectors of ERK1/2, which have divergent physiological effects. The natural product, SL0101, a flavonoid glycoside, binds specifically to RSK1/2 through a binding pocket generated by an extensive conformational rearrangement within the RSK N-terminal kinase domain (NTKD). In modeling experiments a single amino acid that is divergent in RSK3/4 most likely prevents the required conformational rearrangement necessary for SL0101 binding. Kinetic analysis of RSK2 association with SL0101

and its derivatives identified that regions outside of the NTKD contribute to stable inhibitor binding. An analogue with an n-propyl-carbamate at the 4" position on the rhamnose moiety was identified that forms a highly stable inhibitor complex with RSK2 but not with RSK1. These results identify a SL0101 modification that will aid the identification of RSK2 specific inhibitors.

B4. Copper Complexation Strategies for Differentiating Amino Acid Enantiomers by Ion Mobility

Emanuel Zlibut, Jody C. May, and John A. McLean

There has been growing interest in the advancement of efficient and reliable chiral analysis methods that assist with production and quality control of chiral drugs. Current chiral separation techniques require enantiomer specific columns and/or complicated sample pretreatment, which can be expensive and are not ideal for high-throughput analyses. The pursuit of faster, more sensitive analytical techniques for quantitative chiral measurements has led to the development of mass spectrometry-based approaches. In this study, we investigate the separation of chiral amino acids(AA) forming novel tri-nuclear copper clusters in the presence of a chiral selector ([(Cu2+)3(D/LX)3(LY)2-5H]+, X= AA, Y= His, Trp, Gln, Pro, Tyr), that can be directly resolved by ion mobility-mass spectrometry (IM-MS) analyses. Significant enantiomer separation was observed in the IM spectra for hydrophobic AAs acids with peak-to-peak resolutions ranging from 0.63 to as high as 1.15. Among the investigated chiral selectors, histidine, followed by tryptophan, provided the best enantioselectivity (highest IM resolutions), suggesting the aromatic structure plays a vital role in forming chiral-specific ion complexes. The chirality of the selector was found to have no significant effect on observed IM separation, with both D- and L- selectors providing similar resolutions. A combination of MS/MS, collisional cross section measurements, and molecular mechanics techniques are being explored in order to investigate the structural differences between resolvable chiral clusters.

B5. Murine Coronavirus Helicase Mutation Contributes to Remdesivir Resistance

Samantha L. Grimes, Alexandra Abu-Shmais, Maria L. Agostini, Jennifer Gribble Bowser, Xiaotao Lu, Andrea J. Pruijssers, and Mark R. Denison

Coronaviruses (CoVs) have caused three outbreaks of severe disease in humans in the past 20 years, including the current global pandemic caused by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2). This pandemic highlights the importance of developing countermeasures against emerging viruses and understanding current treatments for SARS-CoV-2. Currently, remdesivir (RDV), a nucleoside analogue with broad-spectrum activity against human and zoonotic CoVs, is the only FDA-approved antiviral in use to treat COVID-19, the disease caused by SARS-CoV-2. Widespread use of RDV increases the risk for potential resistance pathways to the drug. We previously reported that serial passaging of murine hepatitis virus (MHV) in GS-441524, the parent nucleoside of the prodrug remdesivir, yielded two resistance mutations located in the RNA-dependent RNA polymerase (RdRp). Reverse engineering of these two mutations together in a wild-type background resulted in a partial recapitulation of the resistance phenotype observed in the parental lineage. Four additional nonsynonymous mutations were identified in this resistant lineage, including a substitution mutation in the helicase, a key enzyme in the CoV replication-transcription complex (RTC). Modeling of this mutation locates the substitution near the predicted RNA-binding channel of the helicase. To determine if this mutation contributed to the resistance phenotype observed in passage, we engineered and characterized this helicase mutation. Our data suggest the helicase mutation by itself confers partial resistance to RDV, independent of the previously characterized RdRp mutations. Further, an engineered virus with the helicase mutation plus the RdRp mutations fully recapitulated the resistance phenotype of the passaged virus. This mutation did not confer alterations to replication kinetics. Importantly, the triple mutant was still sensitive to EIDD-2801, another nucleoside analogue currently in phase 2/3 clinical trials for the treatment of COVID-19. Together, these data suggest an important role for the helicase in RDV antiviral activity and expand an understanding of the molecular mechanisms underlying RDV resistance.

B6. MALDI Imaging Mass Spectrometry of Cell Types within Fresh-Frozen Bone Tissue

Christopher J. Good, Elizabeth K. Neumann, Casey E. Butrico, James E. Cassat, Richard M. Caprioli, and Jeffrey M. Spraggins

Bone and bone marrow are vital to mammalian structure, movement, and immunity. These tissues are commonly subjected to pathological alterations giving rise to debilitating diseases like rheumatoid arthritis, osteoporosis, osteomyelitis, and multiple myeloma. Technologies such as matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) can be leveraged for understanding the complex biological and molecular processes underlying pathology. Preparation of native osseous tissue for MALDI IMS is difficult due to its mineralized composition and heterogenous morphology. Here, we advanced sample preparation techniques to enable multimodal MALDI IMS of fresh-frozen murine femurs at cellular spatial resolution. In brief, adhesive-bound bone sections were mounted flat onto ITO coated glass slides with a microscopy-compatible glue and freeze-dried to minimize bone marrow damage. Subliming matrix was determined to not induce bone marrow cracks, and recrystallizing the deposited matrix improved lipid signal. We report phosphatidylcholine lipid distributions in bone marrow, adipose tissue, marrow adipose tissue, and muscle. Tissue and cell assignments were made by complementary and compatible microscopies. In sum, [sphingomyelin(42:1) + $H]^+$ was determined to be abundant in megakaryocytes, whereas [sphingomyelin(42:2) + $H]^+$ was diminished in the cell. These data allude to the vast molecular and cellular heterogeneity indicative of the bone marrow.

C. SYSTEMS ANALYSIS

C1. Comparative multiplexed interactomics of SARS-CoV-2 and homologous coronavirus nonstructural proteins

Jonathan P. Davies, Katherine M. Almasy, Lars Plate

Human coronaviruses (CoVs) are a threat to global health and society, as evident from the SARS outbreak in 2002, the MERS outbreaks in 2012 and 2014, and the most recent COVID-19 pandemic. Despite the sequence similarity between severe disease-causing CoVs, each strain has distinctive virulence. A better understanding of the basic molecular mechanisms mediating changes in virulence is needed. Here, we profile the virus-host protein-protein interactions of three CoV nonstructural proteins (nsps) that are critical for virus replication. We use tandem mass tag-multiplexed quantitative proteomics to sensitively compare and contrast the interactomes of nsp2, nsp3, and nsp4 from three severe disease-causing CoV strains (SARS-CoV-2, SARS-CoV, MERS-CoV) and two common-cold CoVs (hCoV-OC43, hCoV-229E). This approach enabled us to identify both unique and shared host cell protein interactors and quantitatively compare the enrichment of interactions between homologs. Both nsp2 and nsp4 common interactors are strongly enriched for proteins localized at ER-mitochondria contact sites, suggesting a new functional role for modulating host processes such as calcium homeostasis and mitochondrial function. Nsp3 homologs showed more strain-specific interactor profiles, including interactions with nuclear import machinery for hCoV-229E and ribosomal RNA processing for MERS-CoV. Lastly, we found that the N-terminal fragment of SARS-CoV-2 nsp3 interacts with ATF6, a regulator of the Unfolded Protein Response, and can suppress the ATF6 stress response. Our results shed light on the role these CoV proteins play in the infection cycle, as well as host factors that may mediate the divergent pathogenesis of common cold CoVs from SARS/MERS strains. Our mass spectrometry workflow enables rapid, robust comparisons of multiple bait proteins and identification of common CoV-host dependencies to be targeted by host-directed anti-viral therapeutics.

C2. Molecular Chaperone Recognition of F508del CFTR Unfolding

Eli McDonald, Carleen Sabusap, Lars Plate

Cystic Fibrosis (CF) is a lethal genetic disease caused by mutations in the Cystic Fibrosis Transmembrane conductance Regulator (CFTR). The most common CF patient mutation ΔF508 CFTR folds to a near-native state with properly folded domains, but is thermodynamically unstable due to impaired domain-domain assembly. Molecular chaperones recognize ΔF508 CFTR as thermodynamically unstable and coordinate premature degradation1. Stabilizing ΔF508 CFTR is the only clinically approved, etiologically targeted treatment for CF and is accomplished with small molecules called correctors2. Correctors stabilize ΔF508 CFTR sufficiently to change molecular chaperone recognition1, yet, how ΔF508 gives rise to molecular chaperone recognition remains unclear. We developed a chemical biology approach for identifying molecular chaperones interactions on a domain and subdomain basis using affinity purification mass spectrometry. We site-specifically incorporate photo-crosslinking amino acids into different domains and subdomains in CFTR to determine which domain are unfolded in ΔF508 and subsequently recognized my molecular chaperones. The photo-crosslinking groups covalently capture interacting proteins. We can then identify and quantify these proteins using Tandem Mass Tag (TMT) labeling. Each TMT label gives a sample a unique quantitative signature that allows us perform pair wise statistical analyses between cross linked and non-crosslinked samples. This allows us to quantitatively compare relative site-specific chaperone binding between WT and ΔF508 CFTR. Our method reveals distinct protein interactions for WT and ΔF508 CFTR. Specifically, we show preferential binding of Hsp90 to ΔF508 as shown in previous studies3. Additionally, we show distinct binding partners at nearby sites where the non-canonical amino acid was incorporated. Site specific incorporation of photochemical crosslinkers into CFTR can covalently capture protein interactions identifiable and quantifiable by AP-MS. This method allows for quantitative distinction of chaperones involved in evaluating ΔF508 stability compared to WT in live cells.

C3. The Function of a Bacterial Lytic Transglycosylase in Mitigating Zinc Dysregulation

Jeanette M. Miller, Erin R. Green, Matthew J. Munneke, & Eric P. Skaar

Human coronaviruses (hCoV) have become an increasing threat to global health and society, as evident from the SARS outbreak in 2002 caused by SARS-CoV-1 and the most recent COVID-19 pandemic caused by SARS-CoV-2. Despite the high sequence similarity between SARS-CoV-1 and -2, each strain has distinctive virulence. A better understanding of the basic molecular mechanisms mediating changes in virulence is needed. Here, we profile the virus-host protein-protein interactions of two CoV non-structural proteins (nsps) that are critical for virus replication. We use tandem mass tag-multiplexed quantitative proteomics to sensitively compare and contrast the interactome of nsp2 and nsp4 from three beta coronavirus strains: SARS-CoV-1 from the 2002 outbreak, SARS-CoV-2 from the 2019-2020 pandemic, as well as CoV-OC43 –an endemic strain associated with the common cold. This approach enabled us to identify both unique and shared host cell protein binding partners and further compare the enrichment of common interactions across homologs from related strains. We identified common nsp2 interactors involved in endoplasmic reticulum (ER) Ca^{2+} signaling and mitochondria biogenesis, including the ERLIN1/2 complex and STOML2. We also identified nsp4 interactors unique to each strain, such E3 ubiquitin ligase complexes for SARS-CoV-1, ER homeostasis factors for SARS-CoV-2, and an oxidative-stress pathway component for CoV-OC43. Common nsp4 interactors include N-linked glycosylation machinery, unfolded protein response (UPR) associated factors, and anti-viral innate immune signaling factors. Both nsp2 and nsp4 interactors are strongly enriched in proteins localized at mitochondrial-associated ER membranes suggesting a new functional role for modulating host processes, such as calcium homeostasis, at these organelle contact sites. Our results shed light on the role these CoV proteins play in the infection cycle, as well as host factors that may mediate the divergent pathogenesis of OC43 from SARS strains. Our mass spectrometry workflow enables rapid, robust comparisons of multiple bait proteins, which can be applied to additional viral proteins. Furthermore, the identified, common host-dependencies may present new targets for exploration by host-directed anti-viral therapeutics.

C4. Elexacaftor/VX-445-mediated CFTR interactomics remodeling of misfolding mutations

Minsoo Kim, Carleen M. Sabusap, Eli F. McDonald, Lars Plate

Cystic fibrosis (CF) is one of the most prevalent lethal genetic diseases that has over 2000 identified genetic variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Pharmacological chaperones such as lumacaftor (VX-809), tezacaftor (VX-661) and elexacaftor (VX-445) combat mutation-induced defects by stabilizing the structure of CFTR. The stabilization enables proper folding and thus facilitates processing and trafficking to increase the amount of functional CFTR on the cell surface. Yet, mutant variants display differential correction of CFTR by VX-809. Here, we have identified differential VX-445 response in CFTR variants and investigated the underlying cellular mechanisms of how CFTR biogenesis is altered in these variants. We employed affinity purification-mass spectrometry (AP-MS) multiplexed with isobaric mass tags to define the remodeling of CFTR proteostasis network in CFTR mutant variants in response to correctors such as VX-445. We identified several dysregulated pathways in the CFTR interactome in misfolding variants. VX-809 treatment mitigated the increased interaction between CFTR and proteostasis factors in these pathways. However, in a particular mutant, VX-445 did not correct these interactions and interactome resembled that of control. We narrowed down several proteins as novel targets of interest for functional validation. We found that a rare CFTR mutant variant differentially responds to VX-445. This corrector, unlike VX-809 or VX-661, does not modulate some of the aberrant pathways that leads to misfolding of CFTR. Our results could provide a better understanding of VX-445 biological mechanism of action and reveal novel cellular targets for therapeutic approaches.

C5. Plasma Proteomics to Study Disparities in Sepsis

Kathryn L. Kapp, Sachin Yende, John A. Kellum, Derek C. Angus, Octavia M. Peck-Palmer, & Renã A. S. Robinson

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection. Cellular and metabolic abnormalities substantially increase when persons progress to septic shock. As early as the point of

emergency department admission, patients who will survive sepsis can be differentiated from those who will not survive. However, patient response to sepsis is highly heterogeneous. For instance, hospital data from several decades show African American/Black patients have twice the incidence of sepsis and severe sepsis than non-Hispanic White patients and have a higher rate of hospitalization and mortality due to severe sepsis. African American/Black patients are an average of 9-10 years younger than non-Hispanic White patients when hospitalized with sepsis. Socioeconomic factors contribute to this disparity but do not fully explain it. Therefore, our goal is to analyze a diverse cohort of sepsis patients in order to understand the effects of race on survivorship outcomes on a molecular level. A cohort (N=273) of blood plasma samples from African American and Non-Hispanic White survivors and non-survivors (4 study groups) were obtained from the Protocolized Care for Early Septic Shock cohort at emergency department admission. The cohort was further stratified by primary infection source, intra-abdominal wounds or urinary tract infections. Plasma samples were immunodepleted, digested using trypsin/Lys-C, tagged with tandem mass tag 16-plex (TMTpro) reagents, and subjected to high pH reverse-phase fractionation. Fractions were then analyzed with high-resolution liquid chromatography-tandem mass spectrometry (LC-MS/MS). Data were processed using Proteome Discoverer 2.3 and have been analyzed in a randomized, blinded fashion. Quality control assessments at multiple steps throughout the workflow indicated the data was of high quality and procedures were reproducible day-to-day and sample-to-sample. In total, proteomics analysis identified 2,104 unique proteins. Of these proteins, 471 are quantifiable in all TMTpro channels and are primarily involved in liver X receptor-retinoid X receptor (LXR/RXR) activation, acute phase response, complement and coagulation systems, and farnesoid X receptor-retinoid X receptor (FXR/RXR) activation. All of these pathways have previously been implicated in sepsis. This presentation will describe preliminary results obtained from this study, including the use of quality control assessments throughout the proteomics workflow, as well as implications for understanding outcomes in sepsis patients. This work is the first proteomic study to examine survivor/non-survivor and racial differences in human sepsis.

C6. A *Bacillus anthracis* RNA binding protein post-transcriptionally regulates two component signaling through RNA turnover

Hualiang Pi, Andy Weiss, Clare L. Laut, Caroline M. Grunenwald, Hannah K. Lin, Xinjie I. Yi, Devin L. Stauff, and Eric P. Skaar

Accessory proteins of bacterial two component systems (TCSs) are increasingly appreciated as being involved in the regulation of signal transduction, however, few TCS accessory proteins have been discovered. The HitRS TCS detects and responds to cell envelope damage in the human pathogen *Bacillus anthracis*. Here we report the discovery of KrrA, an RNA binding protein that plays a critical regulatory role in HitRS activation through modulating mRNA stability. KrrA binds to many RNA transcripts including *hitRS*, exhibits no detectable nuclease activity *in vitro*, and potentially functions as an RNA chaperone that facilitates mRNA turnover through assisting the recruitment of the RNA degradosome. Additionally, our data demonstrate that KrrA functions as a global posttranscriptional regulator, reshapes the transcriptome in response to changes in the environment, and controls critical bacterial processes including competence, sporulation, and germination. This study elucidates a regulatory mechanism of TCS signaling and provides insights into bacterial posttranscriptional gene regulation.

C7. In-Virtro Diagnostic Research (IVDr) NMR Spectroscopy at Vanderbilt University

Donald F. Stec and Markus Voehler

Biological fluids (ex. urine, plasma, cerebrospinal fluids) play an important role in both basic scientific research as well as modern health care. These fluids contain essential components called metabolites which can be thought of as "fingerprint" evidence of the breakdown of components that are naturally found in the human body or introduced externally and can often indicate disease states. The ability to accurately identify and quantify these metabolites is referred to as profiling and it is crucial to many areas of basic science and health care such as detection, diagnosis, treatment and prevention of diseases. Lipoprotein main fractions (ex. triglycerides, cholesterol, phospholipids) are also directly related to diagnosis and prevention of health problems. The ability to detect and quantify lipoprotein fragments in plasma is both essential and challenging. Bruker Biospin's new In-Vitro Diagnostics research (IVDr) platform has the ability to determine and quantify detailed lipid profiles and plasma, serum, and urine composition/quantification of 50-80 endogenous metabolites through NMR spectroscopy. The spectrometer is carefully calibrated which enables standardization, quantification and automated data analysis to be provide for body fluid analysis by NMR. Data analysis is supported by a database containing a vast number of compounds which are typically found as metabolites in bodily fluids. The data is stored in a special compressed format which reduces data size and allows for fast searching as well as simultaneous handling of large number of NMR spectra. The data is also saved in a format that allows it to be re-analyzed for future use depending on whether new targets of interest are established or if other applications for the data are discovered. Data transferability and reproducibility is also possible using the IVDr platform since the sample preparation, data collection and analysis are all standardized.

D. THERAPEUTICS AND TRANSLATION

D1. Disrupting host-pathogen protein-protein interactions using host-targeted protein folding regulators

Katherine Almasy, Jonathan Davies, Lars Plate

The COVID-19 pandemic has highlighted the importance of rapidly responding to emerging pathogens. The use of Remdesivir, initially investigated as a broad-acting RNA virus therapeutic, as a treatment for SARS-CoV-2 shows the advantage of having existing antiviral drugs which may potentially be used against future outbreaks. However, several classes of viruses have no current therapeutic options available; among these are the flaviviruses, which include such members as dengue, Zika, and yellow fever. Though several direct-acting antiviral compounds against these pathogens have been examined, many of these ultimately fail due to the development of resistance by the viral genome. We and others propose that instead of directly targeting viral proteins, another option is to target host processes required for the viral life cycle and disrupt macromolecular interactions to block viral replication. To this end, we have shown the ability of compounds 147 and 263, initially designed to assist in host protein folding pathways, to act as antivirals against different strains of dengue and Zika virus. Mechanistic investigations of 147 have shown that the molecule likely causes perturbation of the structure of progeny virions after initial infection, thus affecting their ability to further infect cells. This occurs via covalent modification of cysteine residues on host proteins, and quantitative mass spectrometry approaches for target ID combined with RNAi screening of potential targets indicate that the combined modification of several (largely) endoplasmic reticulum resident proteins is likely responsible for the effect. Viral titers are lowered up to 99% across all strains tested, excitingly showing the potential of these host-targeted molecules to a) be used against several existing viruses and b) be starting points for future drug development against similar pathogens yet to come.

D2. DnaJ and ClpX are required for two-component system signaling in Bacillus anthracis

Catherine S. Leasure, Clare L. Laut, Hualiang Pi, Sophia M. Carlin, Michelle L. Chu, Gideon H. Hillebrand, Hannah K. Lin, Xinjie I. Yi, Devin L. Stauff, and Eric P. Skaar

In bacteria, stress-induced changes in gene expression can occur via signal transduction pathways known as two component systems (TCS). Often, the same stresses that activate TCS, such as membrane disruption, have the potential to interfere with TCS signaling. Thus, bacteria must evolve mechanisms to maintain TCS signaling in the face of this damage. The Gram-positive organism Bacillus anthracis is the causative agent of anthrax. The high rates of mortality associated with anthrax and the potential for use of B. anthracis as a bioterror weapon underscores the risk this organism poses to human health. To survive within the human host, B. anthracis relies on TCS signaling to sense and respond to host- induced stressors. Two important TCS include the cross-regulating TCS HitRS and HssRS which respond to cell envelope disruptions and high heme levels, respectively. In this study, an unbiased and targeted genetic selection was employed to identify gene products that are important for maintenance of HitRS and HssRS signaling. The selection identified DnaJ and ClpX as regulators of TCS signaling. DnaJ and ClpX are the substrate-binding subunits of the DnaJK protein chaperone and ClpXP protease, respectively. DnaJ regulates the protein levels of HitR and HitS to facilitate signal transduction, while ClpX specifically regulates HitS abundance. Together, these results reveal that the protein homeostasis regulators, DnaJ and ClpX, function to maintain B. anthracis signal transduction activities through TCS signaling.

D3. Dominant Negative Effects of *SCN5A* Missense Variants

Matthew J. O'Neill, Ayesha Muhammad, Lynn D. Hall, Joseph F. Solus, Laura Short, Dan M. Roden, Andrew M. Glazer

Up to 30% of patients with Brugada Syndrome (BrS) carry loss of function (LoF) variants in the cardiac sodium channel gene SCN5A. Recent studies have suggested that the protein product $Na_V1.5$ can form dimers, suggesting the possibility that LoF variants may generally have dominant negative effects. We identified 35 LoF variants (<10% peak current compared to wildtype) and 15 Partial LoF variants (10-50% peak current compared to WT). HEK293T cells were engineered to stably express one or two copies of SCN5A using AttB-AttP landing pad and Sleeping Beauty transposon systems. Cell lines were created expressing SCN5A variants alone or in heterozygous co-expression with WT SCN5A. Channel expression was validated by flow cytometry and peak sodium currents were measured using automated patch clamp (>20 replicate cells/variant). We repeated this process for 24 LoF variants expressed in trans with the common polymorphism H558R of SCN5A. To assess clinical risk, we compared the prevalence of dominant negative vs. putative haploinsufficient (frameshift/splice site) variants in a published BrS case consortium and the gnomAD population database. Using automated patch clamping, we assessed the peak current consequences of these variants. WT alone was normalized to 100% and WT+WT showed ~200% peak current when measured at -20 mV. In heterozygous expression with WT, 32/35 LoF variants showed reduction to <75% of WT-alone peak current, demonstrating a dominant negative effect. Interestingly, 6/15 Partial LoF showed similar peak current abrogation. Dominant negative effects were not largely rescued by the co-expression of the H558R allele. To test the hypothesis that dominant negative variants carry a higher disease risk than putative haploinsufficient variants, we compared BrS case vs. control frequencies. We observed a 2.2-fold enrichment for BrS cases among carriers of dominant negative missense variants compared to putative haploinsufficient variants (p=0.048). Most SCN5A missense LoF variants have a dominant negative effect. A common SCN5A polymorphism did not substantially alter this effect. Case-control comparisons reveal that this class of variant carries a disproportionate BrS risk compared to other classes of pathogenic variants.

D4. Regulation of heme biosynthesis by enzymatic structural perturbations

Jenny Tran, Breann Brown

Heme is an essential cofactor involved in numerous biological processes, ranging from oxygen transport to cellular differentiation, and heme biosynthesis must be carefully regulated to avoid physiological issues stemming from nonfunctional heme-free proteins or toxic heme intermediates. The first enzymatic step of heme biosynthesis is widely considered to be the rate-limiting step of the process and is catalyzed by 5'-aminolevulinic acid synthase (ALAS). An important structural feature found in eukaryotic ALAS is the C-terminal extension (CT-ext), in which known mutations lead to human diseases. To characterize the structure and function of the ALAS CT-ext. of *Saccharomyces cerevisiae*, spectrophotometric activity assays were performed, and a crystal structure of *S. cerevisiae* ALAS lacking the CT-ext (Hem1ΔCT) is presented. Our data provides further evidence of a role of the CT-ext in maintaining enzymatic activity of eukaryotic forms of ALAS, thus deepening our understanding of heme biosynthesis regulation.

D5. Antibiotic adjuvants: rejuvenating our current arsenal of antimicrobials against Group B *Streptococcus*

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

As the instance of hospital-acquired bacterial infections rise, antibiotic resistance has become an increasingly urgent threat to healthcare in the United States. While a multitude of bacterial species contribute to this ever-growing problem, our efforts focus on *Streptococcus agalactiae* (Group B *Streptococcus*, GBS). GBS is a gram-positive, opportunistic pathogen colonizing the urinary, reproductive, and gastrointestinal tracts of pregnant mothers. When vertically transmitted from mother to child during birth, GBS infections continue to be the major cause of neonatal sepsis, meningitis, and pneumonia. Current health guidelines suggest prophylactic antibiotic treatment leading up to birth to combat any bacterial transmission, and while previously effective, the rising prevalence of antimicrobial resistance threatens this strategy. In the Townsend and Gaddy labs, we are interested in considering alternative approaches to potentiate antimicrobial efficacy of current treatment options in the form of antibiotic adjuvants. These typically possess no clinically relevant efficacy against a bacterium alone. When combined with known antimicrobials, they increase efficacy by offering rescue mechanisms allowing the antibiotic to circumvent bacterial resistance. An antibiotic adjuvant can be found in any chemically relevant compound, yet in a society wary of medical interventions we are interested in the discovery of these

adjuvant compounds in readily available, widely trusted, household therapeutics. In recent studies we have evaluated several household medicines and have demonstrated their ability to potentiate antimicrobial efficacy against GBS. It is our hypothesis that through these observations and further exploration, supplementation of antibiotic strategies with our adjuvant compounds will alleviate the immediate stress on the ever-serious antimicrobial resistance crisis.

D6. Antibacterial and antibiofilm activity of the human breast milk glycoprotein lactoferrin against Group B *Streptococcus*

Jacky Lu, Jamisha D. Francis, Miriam A. Guevara, Ryan S. Doster, Alison J. Eastman, Lisa M. Rogers, Kristin N. Noble, Shannon D. Manning, Steven M. Damo, David M. Aronoff, Steven D. Townsend, Jennifer A. Gaddy

Group B *Streptococcus* (GBS) is an encapsulated gram-positive human pathogen which causes invasive infections in pregnant hosts and neonates, as well as immunocompromised individuals. Colonization of the human host requires the ability to adhere to mucosal surfaces and circumnavigate the nutritional challenges and antimicrobial onslaught associated with the innate immune response. Biofilm formation is a critical process to facilitate GBS survival and establishment of a replicative niche in the vertebrate host. Previous work has shown that the host responds to GBS infection by producing the innate antimicrobial glycoprotein lactoferrin, which has been implicated in repressing bacterial growth and biofilm formation. Additionally, lactoferrin is highly abundant in human breast milk and could serve a protective role against invasive microbial pathogens. Our work demonstrates that human breast milk lactoferrin has antimicrobial and anti-biofilm activity against GBS and inhibits GBS adherence to human gestational membranes. Together these results indicate that human milk lactoferrin could be utilized as a prebiotic chemotherapeutic strategy to limit the impact of bacterial adherence and biofilm formation on GBS-associated disease outcomes.

D7. A bacterial dehydrin promotes pathogen desiccation tolerance

Erin R. Green, Joseph Fakhoury, Andrew J. Monteith, Hualiang Pi, David P. Giedroc, Eric P. Skaar

The emerging bacterial pathogen *Acinetobacter baumannii* is a leading cause of hospital-acquired infections, and is found ubiquitously on hospital surfaces, which are considered a major reservoir for its transmission. Moreover, the COVID-19 pandemic has caused disruptions to standard infection control practices within hospitals, leading to recent surges in *A. baumannii* cases and underscoring the potential for future outbreaks with this pathogen. *A. baumannii* tolerates an onslaught of stresses encountered during persistence on environmental surfaces, including water loss due to extended periods of dryness, a process termed desiccation. However, the mechanisms underlying the extreme desiccation tolerance of *A. baumannii* and the impact of desiccation on pathogen transmission and infection severity remain largely unexplored. Here, we show that *A. baumannii* causes more virulent infections following rehydration from desiccation and we uncover a mechanism dictating desiccation tolerance in this pathogen. We report the discovery of the first bacterial dehydrin, the intrinsically disordered protein (IDP), DtpA, which promotes desiccation tolerance in *A. baumannii.* Recombinant DtpA is sufficient to protect purified enzymes from desiccation and heat inactivation, and heterologous expression extends the desiccation tolerance of a probiotic bacterium, indicating a function for DtpA in the preservation of proteins during denaturing conditions. Additionally, we show that DtpA expression is controlled by a conserved protease, revealing a key regulatory mechanism utilized by *A. baumannii* to tolerate environmental stress. In summary, our results uncover a previously unknown connection between environmental persistence and pathogenicity in *A. baumannii* and provide a mechanism contributing to the extreme desiccation tolerance of this organism. Furthermore, these findings highlight a previously unexplored role for IDPs in bacterial stress tolerance and infection, and reveal potential applications for bacterial dehydrins to preserve activity of protein- and live bacteria-based pharmaceuticals that require desiccation for long term storage and transport.

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