Scientific Sessions: SLC Board of Trust Room

Poster Session / Reception / Science Jeopardy:
Engineering Science Building Lobby

CSB Symposium 2024 Program of Events

Vanderbilt University

[Interactive Campus Map]

Student Life Center Board of Trust Room:

Welcome

8:30 – 8:50 **Coffee and pastries**

8:50 – 9:00 **Opening Remarks**

Scientific Session #1

Session 1A – Moderator: Vida "Storm" Robertson, Damo Lab

9:00 – 9:45 **Nozomi Ando, Cornell University**

Combining structural techniques to study protein motions

9:45 – 10:00 **Doug Kojetin, Vanderbilt University**

Nuclear receptor function made crystal clear with NMR spectroscopy

10:00 – 10:15 **Break**

Session 1B – Moderator: Katherine Stefanski, PhD, Sanders Lab

10:15 – 11:00 **James Fraser, UCSF**

Discovering new ligands for viral macrodomains with fragments, AI, and entropy!

11:00 – 11:15 **Tina Iverson, Vanderbilt University**

A structural basis for bacterial chemotaxis

Session 1C – Moderator: Chris Williams, Kojetin Lab

11:15 – 11:30 **Selected Abstract Talk: Ana Chang-Gonzalez, PhD, Meiler Lab**

Classification of nuclear receptor LRH-1 activity in cell-based assays using RosettaLigand energy terms

Session 1D – Moderator: Shannon Kordus, PhD, Lacy Lab

11:30 – 12:15 **Jeanne Hardy, University of Massachusetts, Amherst**

Harnessing protease reactions for detection and treatment of viral infection

12:15 – 12:30 **Breann Brown, Vanderbilt University**

Structure-Based Insights into the Regulation of Heme Biosynthesis

Lunch

12:30 – 1:30 **Box lunches available**

Scientific Session #2

Session 2A – Moderator: Amy Tran, Meiler Lab

1:30 – 2:15 **Andrej Sali, UCSF**

From integrative structural biology to cell biology

2:15 – 2:30 **Will Wan, Vanderbilt University**

Characterizing the molecular mechanisms of Ebola virus budding

2:30 – 2:45 **Break**

Session 2B – Moderator: Chris Williams, Kojetin Lab

2:45 – 3:00 **Selected Abstract Talk: Rong Sun,PhD, Zhou Lab**

Unveiling ferrosome formation by cryo-electron tomography

Session 2C – Moderator: Cameron Cohen, Jackson Lab

3:00 – 3:30 **Poster Flash Talks**

Closing

3:30 – 3:40 **Closing Remarks**

Engineering & Science Building Lobby:

Networking Events

3:45 – 5:00 **Poster Session** and **Reception**

5:00 – 6:00 **Science Jeopardy Game**: *Hosted by Ray Blind*

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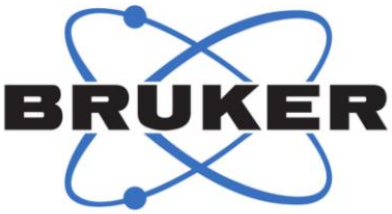

CSB Symposium 2024 Poster Session

POSTER #1

Epitope-focusing and viral escape prediction for the design and selection of robust influenza antibodies

Gustavo Araiza[1], Cristina E. Martina[1], Brian O. Bachmann[1], James E. Crowe[2], Jens Meiler[1]

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Influenza A viruses account for 650,000 deaths annually, worldwide. Therapeutic antibodies have a high potential to act as prophylactic agents against viruses. Here, we take advantage of a novel antibody, H7-200, that binds the influenza surface protein hemagglutinin across a cryptic and conserved epitope known as the TI-II. We use ProteinMPNN and Rosetta to retarget H7-200 towards different HA subtypes, as the TI-II has only been found along the H7 subtype.

POSTER #2

Molecular modeling of integrin modification during diabetic hyperglycemia and carbonyl stress

Eric W. Bell, Benjamin P. Brown, Ambra Pozzi, Jens Meiler

Departments of Chemistry, Pharmacology, and Biomedical Informatics, Center for Structural Biology, and Institute of Chemical Biology, Vanderbilt University

A common cause of complications in diabetes is the modification of protein residues by spontaneous glucose attachment (glycation) and carbonyl stress. These stresses result in the appearance of modified residue types, such as carboxymethyllysine (CML) and hydroimidazolone (MG-H1). The modification of residues can interrupt the typical function of proteins such as integrins, which adhere cells to the extracellular matrix. In order to study how modified residues impact integrin function, we first modeled the structure of two integrins (alpha1beta1 and alpha2beta1) using AlphaFold Multimer, and modeled the impact of mutating each lysine/arginine residue to CML/MG-H1 on the protein using the non-canonical amino acid parameterization tools of Rosetta. Through this procedure, we identified several residues of interest which could have significant impact on integrin stability if they were to be modified through glycation and carbonyl stress. In addition, we present a brief benchmark of non-canonical amino acid modeling in Rosetta as well as the first steps toward graph convolutional neural network prediction of glycation, which will complement our mutational scanning by determining which residues are most likely to be modified.

POSTER #3

Intertwined dimer of human PTK6 SH3 domain

Berndt S., Buhl L., Kupsch L., Oeguetcue D., Rooss K., Liebscher I.

Rudolf-Schoenheimer Institute of Biochemistry, Signal Transduction, Medical Faculty, Leipzig, Germany

Protein tyrosine kinase 6 (PTK6, or breast tumor kinase, Brk) is abundant in multiple tumor types like breast and prostate cancer. PTK6 expression levels correlate with tumor grade and invasiveness. There is only limited structural and functional data of PTK6 available. PTK6 is closely related to Src family kinases, containing functional domains (SH3, SH2, and kinase domain). PTK6 mediates regulation of multiple signaling pathways through Src kinase, MAPK p38 and ERK 5 activation. Here we present a novel intertwined dimer crystal structure of the human PTK6 SH3 domain at 1.75 Å. The dimerization process results in a domain swap architecture, which is a result of a distinctive dimerization mechanism. We found that the dimerization is inducible in vitro and observed the dimerization in cells, which indicates a potential regulatory role of dimerization in cells.

POSTER #4

Elucidating the Type IV Secretion System-Mediated Delivery of a Bacterial Oncoprotein

Kaeli N. Bryant[1], Mark S. McClain[2], and Timothy L. Cover[1,2,3]

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[3]Veterans Affairs Tennessee Valley Healthcare System

Virulent strains of *Helicobacter pylori* deliver CagA (designated as a bacterial oncoprotein) into host cells through actions of the Cag type IV secretion system (T4SS), thereby contributing to the pathogenesis of gastric cancer. I found that a GFP-CagA fusion protein interacts with other components of the Cag T4SS but is not delivered into host cells. I hypothesize that the GFP moiety obstructs T4SS-mediated delivery of CagA and other substrates into host cells.

POSTER #5

Membrane remodeling by endosomal coat complexes

Mintu Chandra[1,2], Amy K. Kendall[1,2], and Lauren Jackson[1,2]

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[2]Center for Structural Biology, Vanderbilt University

Coordinated movement of proteins and lipids between different organelle compartments is fundamental to cell biology and human health. Trafficking pathways enable diverse physiological processes, including nutrient uptake, synaptic transmission, signal transduction and activation of the immune response to invading pathogens. Many trafficking genes are essential for organism viability, while others are affected in a variety of acquired and genetic diseases, such as cancers and neurodegenerative diseases. Specific coat protein complexes define different pathways and ensure critical cargoes are directed to the correct destination in a timely manner. Retromer, a heterotrimeric protein complex consisting of VPS26, VPS29, and VPS35, has been known to couple with different Sorting Nexins (SNXs) to form multiple endosomal coat complexes to traffic large number of transmembrane cargoes. Accessory proteins like VARP, Rab7, TBC1D5, and the WASH complex interact with metazoan Retromer, influencing endosomal dynamics.

In our recent study, we used biochemical reconstitution approaches to explore multiple combinations of endosomal coat proteins on liposomes in the presence of relevant phospholipids and cargo motifs. We paired liposome pelleting assays with negative-stain electron microscopy (EM) to ascertain conditions under which combinations of SNX and Retromer proteins can bind and tubulate membranes. Here we demonstrate for the first time how metazoan SNX27 on its own and together with Retromer can deform and tubulate membranes enriched with PI(3)*P* and SNX27 cargo motifs (PDZbm). We further show how the SNX-BAR heterodimer SNX2/SNX6 (also called ESCPE-1) can deform and tubulate membranes enriched with PI(4,5)P_2 and CI-MPR cargo motifs. These two different endosomal coats yield tubules having different physical diameters. We tested whether SNX2/SNX6 can engage SNX27/Retromer to form the proposed ‘supercomplex’, and we find supercomplex formation depends on the presence of VARP in our reconstitution system. Biochemical pulldown assays, quantitative biolayer interferometry (Blitz) experiments and comprehensive AlphaFold Multimer analyses demonstrate that VARP interacts directly with SNX27 with micromolar affinity. Altogether, these results advance a molecular understanding of how different endosomal coat components generate transport carriers for the efficient sorting of cargoes from the endosomes to plasma membrane potentially via the formation of ‘supercomplex’.

POSTER #6

Molecular mechanisms of pathogenic missense variants in the active site of the hepatic glucose-6-phosphatase catalytic subunit (G6PC1)

Matt Sinclair[1,2], Richard A. Stein[3,4], Jonathan H. Sheehan[5,6], Emily M. Hawes[3], Richard M. O'Brien[3], Emad Tajkhorshid[1,2,7], and Derek P. Claxton[3,4.5]

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A long-term goal of the Claxton research program is to illuminate structural and mechanistic principles of hepatic glucose-6-phosphatase catalytic subunit 1 (G6PC1), a membrane-integrated endoplasmic reticulum (ER) enzyme implicated in metabolic diseases. A member of the type 2 phosphatidic acid phosphatase (PAP2) superfamily, G6PC1 functions as the gatekeeper of hepatic glucose production (HGP) by catalyzing hydrolysis of glucose-6-phosphate (G6P) to glucose and inorganic phosphate (Pi), the final step of gluconeogenesis and glycogenolysis. Elevated G6PC1 activity contributes to the increased HGP associated with type 1 and type 2 diabetes. Additionally, more than 100 genetic mutations in the *G6PC1* coding region that reduce or abrogate enzyme activity cause glycogen storage disease type 1a (GSD). While its role in metabolic disease makes G6PC1 an attractive therapeutic target, knowledge gaps in the molecular structure and mechanism of hydrolysis and inhibition preclude drug discovery efforts. We have taken an integrative approach that capitalizes on computational predictions with experimental validation to address these shortcomings. Using a high accuracy AlphaFold2-predicted model (pLDDT = 92.58), we determine the atomic interactions of G6P binding to the active site of G6PC1 with molecular dynamics simulations. Nearly half of the coordinating side chains are positions of naturally occurring GSD mutations. Combining robust biochemical and biophysical assays with computational analysis, we find that pathogenic mutations in the active site of G6PC1 induce structural, thermodynamic, and kinetic perturbations that are likely to contribute to disease etiology.

POSTER #7

A High-Throughput Screen to Identify Modifiers of KCNQ1 Trafficking

Katherine R. Clowes[1], Mason C. Wilkinson[1], Katherine M. Stefanski[1], and Charles R. Sanders[1]

[1]Department of Biochemistry, Vanderbilt University

Approximately 1 in 2500 individuals suffer from congenital long QT syndrome (LQTS), a cardiac disorder that can cause syncope, cardiac arrythmia, and cardiac arrest, which can be fatal. Loss-of-function mutations in the voltage gated potassium channel protein KCNQ1 causes type 1 long QT syndrome (LQT1), which accounts for up to 50% of cases of LQTS. Over 250 LQT1-causing mutations in KCNQ1 have been identified, but it is unknown whether there is a common mechanism through which these mutations lead to disease. Previous studies have found that only ~20% of expressed WT KCNQ1 successfully traffics to the plasma membrane, and that many LQT1-associated mutations in KCNQ1 destabilize the protein and decrease trafficking efficiency further. Protein mistrafficking has been identified as a mechanism of several diseases and has been found to be rescuable with small molecules. This led us to hypothesize that mistrafficking is a common mechanism of KCNQ1 loss-of function in LQT1 and that fold-stabilizing small molecules can increase the trafficking efficiency of KCNQ1. To test this hypothesis, we developed an immunofluorescence-based high-throughput assay to identify compounds that alter the trafficking of KCNQ1 in cells. Screening of small molecule libraries has identified several hits that alter the expression and trafficking efficiency of WT and disease-causing mutant forms of KCNQ1. Several small molecules have been identified that reduce the trafficking of “super trafficking” mutant R231C back to WT-like levels. Follow up experiments are ongoing and will determine the specificity and mechanism of action of these compounds, as well as whether they bind and stabilize KCNQ1 directly. These studies will contribute to our larger hypothesis that misfolding induced mistrafficking is a common, rescuable, mechanism of KCNQ1 loss-of-function in LQT1 and inform potential routes for treatment of type 1 long QT syndrome.

POSTER #8

Structural insights into Gβγ-SNARE mediated inhibition of exocytosis

Anna R. Eitel[1] and Heidi E. Hamm[1,2]

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G-protein βγ heterodimers (Gβγ) liberated upon activation of presynaptic inhibitory G-protein Coupled Receptors ($G_{i/o}$ GPCRs) prevent neurotransmission downstream of Ca^{2+} influx through direct interactions with the ternary N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex. The ternary SNARE complex is composed of synaptosomal-associated protein, 25kDa (SNAP-25), syntaxin-1A, and synaptobrevin-2. Previous work from the Hamm lab has shown that Gβγ interacts with all three neuronal SNAREs individually but binds the ternary complex with the highest affinity. Interactions between Gβγ and the C-terminus of SNAP-25 are essential for Gβγ-mediated inhibition, and Gβγ competes with the Ca^{2+}-sensor synaptotagmin for an overlapping binding site on SNAP-25. There is no experimental structural data for this interaction, and the precise molecular mechanism remains poorly understood. To address this, we have expressed and purified a pre-fusion ternary SNARE mimetic containing a C-terminal truncation of synaptobrevin-2 which prevents full zippering of the SNARE complex. This partially zippered SNARE construct has a higher affinity for $G\beta_1\gamma_2$ than the fully zippered version. We stabilized $G\beta_1\gamma_2$-SNARE complexes both with and without complexin using a crosslinker for analysis via mass spectrometry (MS) and cryo-EM. We collected an initial (~700 micrograph) single-particle dataset of $G\beta_1\gamma_2$-SNARE-Complexin samples on a Thermofisher Glacios cryo-TEM and obtained a 5.7 Å density map after processing with cryoSPARC. We are currently building an atomic resolution model combining our single particle and biochemical data with computational docking simulations.

POSTER #9

The S100A9 C-terminal tail of calprotectin modulates metal binding and anti-bacterial activity against *Staphylococcus aureus*

Kyle T. Enriquez*[,1,2,3], Yasiru R. Perera*[4,5], Aslin Rodriguez[4,5], Velia Garcia[4,5], Anais Naretto[4,5], Ryan Guillen[4,5], Tae Akizuki[4,5], Walter J. Chazin[4,5], and Eric P. Skaar[2,3]

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Calprotectin is an S100A8/A9 heterodimer that sequesters trace transition metals at the host-microbe interface. Preliminary results suggest the S100A9 C-terminal tail participates in a unique hexacoordinate binding site. Transition metal availability regulates *Staphylococcus aureus* pathogenesis, and results from growth and Temporal Modelling of the Biofilm Lifecycle assays suggest the S100A9 tail is not essential to anti-bacterial activity of calprotectin in vivo, consistent with growth inhibition dependent on sequestration of zinc in these media, not manganese.

POSTER #10

Navigating the Landscape of Nuclear Receptors: Conformational Ensembles, Evolutionary Insights, and Therapeutic Prospects in PPARG and Nurr1

Mithun Nag Karadi Giridhar[1] and Douglas J. Kojetin[1]

[1]Department of Biochemistry, Vanderbilt University

Nuclear receptors are critical transcription factors governing genes vital for metabolism, immunity, and development. Comprising 48 subtypes with ligand and DNA binding domains, nuclear receptors are prime targets for therapeutic interventions with 20% of FDA approved drugs targeting them. However, challenges due to cross-reactivity, prompt the necessity for developing next-generation small molecules. To address this, a comprehensive understanding of conformational ensembles and high-energy structures in equilibrium becomes imperative. This study integrates biophysical, evolutionary, and computational techniques, focusing on the ligand binding domain of nuclear receptors, specifically, Nurr1 and PPARG from the NR1 and NR4A subfamily. We constructed a comprehensive NR tree from 16,000 sequences revealing the close relationship between NR1 and NR4A subfamilies. Folding studies of PPARG and Nurr1 unveil compelling differences in stability and an ancestrally conserved intermediate for both the enzymes. Significantly, this high-energy intermediate, conserved in the landscape, showcases evolutionary adaptations tailoring the ensemble between the native and intermediate state for distinct regulation of PPARG and Nurr1. A crucial C-terminus mutation in Nurr1 shifts the ensemble toward a more dynamic state observed in PPARG, illustrating how cells could modulate the ensemble for specific pathways. These studies provide pivotal insights, offering a roadmap to navigate the challenges in current therapeutics and paving the way for precision interventions targeting specific confirmations that effect specific pathways governed by nuclear receptors. Embarking on this scientific quest promises not only to expand our understanding but also to unlock the potential for groundbreaking therapeutic strategies.

POSTER #11

Discovery and Characterization of a Pan-betacoronavirus S2-binding antibody

Clinton M. Holt[1,2 *], Nicole V. Johnson[3 *], Steven C. Wall[1,4*], Kevin J. Kramer[1,4*], Sivakumar Periasamy[5,6], Simone Richardson[7,8], Naveenchandra Suryadevara[1], Emanuele Andreano[9], Ida Paciello[9], Giulio Pierleoni[9], Giulia Piccini[10], Ying Huang[11,12], Pan Ge[11], James D Allen[11], Naoko Uno[13,14], Andrea R. Shiakolas[1,4], Kelsey A. Pilewski[1,4], Rachel S. Nargi[1], Rachel E. Sutton[1], Alexandria A. Abu-Shmais [1 4], Robert Parks [15], Barton F. Haynes[15,16], Robert H. Carnahan[1,17], James E. Crowe Jr[1,4,17], Emanuele Montomoli[10,18,19], Rino Rappuoli[9,20], Alexander Bukreyev[5,6], Ted M. Ross[11,13,14,21], Giuseppe A. Sautto[11#], Jason McLellan[3#], Ivelin S. Georgiev[1,4,22,23,24,25#]

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Despite extensive structural characterization of SARS-CoV-2 spike epitopes, the highly conserved S2-region of spike had no previous residue-resolution structures of antibodies bound to conformational protein epitopes in this domain. Here, we discovered an extraordinarily broad anti-coronavirus antibody which provided mice mild protection from disease. We then solved a 3.0 Å resolution cryo-EM structure of its complex to a pre-fusion stabilized S2-construct, describing a cryptic apical epitope buried beneath the S1-domain in the closed pre-fusion state.

POSTER #12

Characterizing the Spatiotemporal Dynamics of VP40 and Intracellular Calcium Influx During Ebola Virus Assembly

Tyler Huth, William Wan

Department of Biochemistry, Vanderbilt University

Ebola virus (EBOV) is an enveloped, single-stranded RNA virus which persists as a re-emerging threat in Central and Western Africa, with outbreaks reporting up to a 90% case fatality rate. A key aspect of EBOV pathogenesis is the assembly and budding of viral particles which is driven by EBOV's matrix protein VP40. VP40 traffics to the plasma membrane, where it oligomerizes, bending the membrane and self-assembling into filamentous viral particles. While VP40 membrane localization and oligomerization are necessary for virus-like particle (VLP) production, studies show that an influx of cellular calcium is also required for efficient viral budding. VP40 expression alone triggers an increase in intracellular calcium concentrations but what aspects of VP40 expression drive this influx is poorly understood. Disrupting calcium influx correlates with reduced budding efficiency, but the mechanism behind calcium-mediated regulation of EBOV VLP egress remains unclear. Understanding how EBOV perturbs cellular calcium homeostasis to promote budding provides valuable insights into the mechanisms of viral assembly, cell responses, and potential avenues for therapeutic interventions.

VP40-driven assembly and budding of VLPs is an inherently dynamical process that cannot be fully characterized by endpoint analysis of fixed cells. We have developed a live-cell imaging approach to capture the real-time spatial and temporal dynamics of the interplay between calcium and budding. We engineered inducible fluorescently-tagged constructs of wild-type VP40 and functionally deficient mutants, enabling visualization of their intracellular dynamics and interactions with host cell membranes. Currently, we are investigating whether calcium influx is contingent upon reaching a critical protein concentration, correlated with the subcellular localization of VP40, or dependent on VP40's oligomeric state. By studying the spatiotemporal dynamics of fluorescently tagged VP40 in relation to calcium biosensors, we aim to decipher the mechanisms that drive the complex interplay between calcium concentration and VP40-driven EBOV assembly.

POSTER #13

The Role of Transmembrane Helices in the Modulation of Epidermal Growth Factor Receptor Activity

Deepti Karandur, Kate Miller, Yongjian Huang, and John Kuriyan

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The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that initiates a variety of cellular processes including cell growth, differentiation, and proliferation. Structurally, EGFR comprises of an extracellular ligand-binding module, a single-pass transmembrane helix and an intracellular catalytic, kinase domain. Activation occurs when a ligand binds to the extracellular module leading to a structural transition that facilitates dimerization. EGFR can bind to seven ligands, all of which activate EGFR by dimerizing the receptor. Yet binding by different ligands elicits vastly different downstream signals. Recent structural studies have demonstrated that the extracellular module can assume a range of dimeric, active conformations and various ligands stabilize these conformations to different extents. Biochemical and cell-based studies have shed light on how this ultimately leads to differential downstream signaling. However, the mechanism by which the conformational state of the extracellular module is communicated to the intracellular catalytic domains via single-pass transmembrane helices is unknown. This is largely due to the highly dynamic nature of the protein in the transmembrane region. Here, we use molecular dynamics simulations and more complex enhanced sampling simulation methods to show that key associations between the extracellular module, the transmembrane helices and the membrane allow certain conformations of the extracellular module to couple to distinct configurations of the transmembrane domains. Mutations that disrupt this coupling abrogate the differential downstream signaling by two ligands – EGF and TGFα. These studies shed light on how EGFR might transition between higher-activity and lower-activity states in response to binding by different ligands and suggest an intriguing mechanism whereby the protein functions in conjunction with the membrane to initiate and regulate various downstream pathways.

POSTER #14

From toxins to capsule to vesicles and back again: understanding how *Clostridioidies difficile* modulates virulence factors

Shannon Lynn Kordus[1,2,3*], Evan Krystofiak[4], Rubén Cano Rodriguez[1,2,3], Kevin Childress[1,2,3], and D. Borden Lacy[1,2,3]

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The nosocomial pathogen *Clostridioides difficile* causes life-threatening diarrhea as a result of the production of two large toxins, TcdA (308 kDa) and TcdB (270 kDa). Understanding toxin secretion in *C. difficile* has not been well studied. Using ultrasensitive reagents, we were able to perform a time course assay in vitro to measure TcdA and TcdB. To our surprise, we found that various strains of *C. difficile* secreted toxins are various time and rates. Specifically, despite making intracellular TcdB, many strains of *C. difficile* delay secreting the toxin. Furthermore, we found a maximum amount of toxin secreted by the bacterium. To explore this phenomone further, we hypothesized that the creation of an additional extracellular virulence factor may prevent toxins from being secreted, specifically by the production of a capsule. We visualized capsule using cryo-SEM and found that capsule biosynthesis appears to begin during time points when the bacteria fail to make increasing amounts of toxin. While growing *C. difficile* in minimal medium to better visualize the capsule, we observed “trapped” membrane vesicles in between the inner membrane and peptidoglycan layer. To explore this phenomenon further, we purified membrane vesicles from capsulated and non-capsulated bacteria and found that capsulated bacteria produced few vesicles compared to non-capsulated bacteria. To determine the contents of the vesicles, we performed a proteomic analysis which revealed that the toxins TcdA and TcdB were found in vesicles associated with non-capsulated bacteria. Currently, toxin secretion is thought to occur via a non-classical secretion system that involves a holin-like protein, TcdE, and a partial endolysin, TcdL. Initial studies indicated that in non-encapsulated strains containing deletions in tcdE, fewer vesicles were observed. Further studies are needed to test for a link between capsule production and vesicles during *C. difficile* infection and whether vesicles play a role in toxin secretion.

POSTER #15

The mechanism of PPARγ transcriptional repression by potential urothelial cancer therapeutic FX-909

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Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-responsive nuclear receptor (NR) transcription factor that plays a role in cell differentiation and metabolism. PPARγ activity is regulated by endogenous fatty acids and lipids, including dietary and fatty acid metabolites, as well as synthetic ligands including FDA-approved antidiabetic drugs that activate transcription of gene programs involved in adipogenesis and insulin sensitization. Recent studies have implicated ligand-induced repression of PPARγ transcription in the treatment of advanced urothelial cancer where PPARγ signaling is hyperactivated. In this project, we are investigating the mechanism of action of a clinical candidate compound called FX-909, which is slated to enter phase 1 clinical trials, using biochemical, structural biology, and cellular methods. We show that FX-909 functions as an inverse agonist of PPARγ to a similar extent as previously characterized compounds and have determined a crystal structure of FX-909 bound to the PPARγ ligand binding domain (LBD), which reveals the ligand covalently binds to a cysteine residue (Cys285) within the orthosteric ligand binding pocket similar to other PPARγ ligands. With these and other data comparing the efficacy FX-909 to other structurally related compounds synthesized by our collaborators, we hope to provide insight into the mechanism of small-molecule transcriptional repression of PPARγ, which could guide drug design of future bladder cancer therapeutics.

POSTER #16

Targeting the Peripheral Myelin Protein 22 using Fragment Based Drug Discovery

Geoffrey Li, Thilini Ukwaththage, Charles R. Sanders

Center for Structural Biology and Department of Biochemistry, Vanderbilt University

Charcot-Marie-Tooth Disease (CMTD) is a hereditary disorder of the peripheral nervous system that affects 1 in 2500 humans, for which there is no available treatment. The most common forms of CMTD are associated with genetic aberrations in the peripheral myelin protein 22 (PMP22), a tetraspan membrane protein that is abundant in compact myelin. PMP22 overexpression, underexpression, or missense mutations result in defective myelin in the peripheral nerves. This is mainly due to misfolding and aggregation of PMP22. In this work, we use NMR spectroscopy to screen a fragment library in search of compounds that bind to PMP22 *in vitro* and potentially impact protein folding and function. We present a description of the methodological development and optimization to apply NMR-based fragment screening to an integral membrane protein target. After screening PMP22 in dodecyl-β-maltopyranoside (DDM) micelles against a subset of the library, we found a number of compounds that bind to the protein in DDM micelles but not to a negative control membrane protein. The binding affinity of the hits were then determined by NMR. Ongoing assessment of analogs of fragment hits demonstrates the feasibility of NMR-based fragment screening to identify chemical matter that binds a tetraspan membrane protein.

Acknowledgements: We thank Dr. Stephen Fesik for the use of the fragment library. This work was supported by Deerfield Management Company, L.P through Ancora Innovation, the Vanderbilt/Deerfield partnership.

POSTER #17

Structural basis of ligand-dependent repression of circadian rhythm transcription factors REV-ERBα and REV-ERBβ

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REV-ERBα and REV-ERBβ are ligand-regulated nuclear receptor (NRs) transcription factors that regulate the expression of gene programs critical for many physiological functions, especially in the regulation of circadian rhythms. REV-ERBs recruit co-repressor proteins NR corepressor (NCoR) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) to chromatin, resulting in repression of gene expression. Previous biochemical and structural studies in the Kojetin lab have shown that the endogenous REV-ERB ligand heme enhances binding of N-CoR to REV-ERBβ LBD. However, due to poor expression level and solubility of REV-ERBα-LBD, it has been challenging to study the heme-dependent REV-ERBα/corepressor interactions. Here, I used a computational algorithm called PROSS to design mutant constructs of REV-ERBα-LBD to generate mutants that could be purified through a laboratory-scale method for biochemical and structural studies. Moreover, although synthetic REV-ERB ligands have been reported for REV-ERBs, the structural basis of synthetic REV-ERB modulation regulation remains poorly understood. With the purified REV-ERBα-LBD, I plan to study how heme and synthetic ligand binding to REV-ERBα LBD regulates co-repressor interaction and function.

POSTER #18

Design and Application of Genetically Encoded EM-visible V-Tag for CryoET

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Cryo-Electron tomography (cryoET) has revolutionized our understanding of biological function by revealing the native molecular details of cells, membranes, and organelles. Despite the advances, the exact localization of biomolecules of interest within the tomographic volumes remains a formidable challenge due to the crowding biological sample environment and low signal-to-noise ratios. To address this challenge, we are developing several novel tags with distinctive shapes and low molecular weights. Among these, the V-shaped tag, validated through high-precision structural prediction software and protein purification techniques, enables us to visualize the proteins or organelles we've labeled within the cellular context.

POSTER #19

Rescuing from the DEAD: Understanding Substrate-Mediated ATP Hydrolysis through Deep Mutagenesis of a DNA Polymerase Clamp Loader

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Clamp loaders are AAA+ ATPases that facilitate processive DNA replication by loading a sliding clamp on primed DNA. This process is reliant on ATP hydrolysis triggered by DNA substrate binding to the clamp loader. The underlying principles that govern substrate-mediated catalysis in AAA+ are still not well understood. Like many other ATPases, T4 bacteriophage clamp loaders contain a highly conserved DExD motif within each active site of the pentameric ATPase. In the distantly related ATPase family, DEAD-box RNA helicases, the final aspartic acid residue of the DEAD motif mediates structural rearrangements necessary to couple RNA binding to ATP hydrolysis. We take inspiration from this mechanism and study a partial loss-of-function mutation to the final aspartic acid of the DExD motif (D110C) in T4 bacteriophage clamp loaders through deep mutagenesis and biochemical assessment. Through a variety of biochemical assays, we find that the mutated (D110C) clamp loader is unable to bind primed DNA and has compromised ATP hydrolysis activity. We then use the D110C clamp loader as the basis for a saturation-mutagenesis experiment that identifies regions in which mutations can rescue phage propagation. The nature of these mutations suggests that intramolecular interactions present in the wild-type protein need to be broken to rescue the activity of the D110C clamp loader. Our data suggest that the DNA-free state of the clamp loader represents a low energy conformation that is converted to the active state by DNA binding. Our results show how deep-mutagenesis experiments can provide valuable insight concerning the mechanisms of complex molecular machines.

POSTER #20

A mechanistic model of primer synthesis from catalytic structures of DNA polymerase α–primase

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The mechanism by which polymerase α–primase (polα–primase) synthesizes chimeric RNA-DNA primers of defined length and composition, necessary for replication fidelity and genome stability, is unknown. We report cryo-EM structures of polα–primase in complex with primed templates representing various stages of DNA synthesis. These structures, along with biochemical and biophysical assays, elucidate a critical catalytic step in replication initiation and provide a comprehensive model for primer synthesis by polα–primase.

POSTER #21

Investigating Critical Motifs for Heme-Dependent Regulation of Erythroid-Specific Aminolevulinic Acid Synthase

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The cytotoxicity of free heme necessitates tight regulation of heme production. Aminolevulinic acid synthase (ALAS) catalyzes the first and rate-limiting step of heme biosynthesis. ALAS has five predicted heme regulatory motifs (HRMs), suggesting a potential route for feedback regulation. Our preliminary UV-Visible spectroscopy data show evidence of heme binding to mature erythroid-specific human ALAS. Future studies will determine which motifs are necessary and sufficient for heme-dependent regulation.

POSTER #22

The thermodynamic stability and misfolding properties of S100A12

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S100A12 is a protein implicated in chronic inflammation, a shared characteristic in cancer and other diseases. S100A12 also forms amyloid in gastric tissue of *H.pylori*-infected mice, an animal model for stomach cancer. To elucidate the mechanism of S100A12 amyloid formation, we incubated S100A12 at low pH to demonstrate it can form amyloid structures. Differential scanning fluorometry experiments confirm that low pH destabilizes S100A12 structure, reducing its melting temperature. To elucidate the pH mediated misfolding protein pathway of S100A12, hydrogen/deuterium exchange experiments are in progress. Together, these preliminary data explore the thermodynamic stability and the misfolding properties of S100A12 to understand the mechanism of S100A12 amyloid formation.

POSTER #23

Small-molecule modulators of protein raft affinity and raft stability

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Lipid rafts remain an active area of membrane biophysics research but due to their nanoscale size and theorized short lifetime their exact cellular functions have yet to be clarified. Giant plasma membrane vesicles, which spontaneously separate into ordered (raft) and disordered (non-raft) phases, are a practical a tool for studying rafts and raft-resident proteins. The lack of tools to manipulate raft-affinity of proteins has stymied our ability to study the functions of lipid rafts in cells. One such protein, PMP22 shows a high affinity for ordered domains (lipid rafts) in GPMVs. Genetic defects in PMP22 cause Charcot-Marie-Tooth disease. Several disease-causing mutations in PMP22 exhibit decreased raft partitioning which correlates with disease severity, but the functional consequences of raft affinity are not understood. Using a high-throughput screening pipeline, we screened 20,000+ small molecules in search of compounds that alter the raft affinity of PMP22. Hits were counter-screened against another raft partitioning protein, MAL. Here, we describe two classes of compounds discovered in this screen. First, we discovered a novel class of compounds which alter both PMP22 raft-partitioning and raft stability. Interestingly, these compounds, which we call global modulators, are not protein specific but are protein-dependent suggesting a cooperativity between lipids and proteins that form rafts. In contrast, we have also characterized small-molecule modulators of raft stability that act independently of protein content. We also show these compounds have differential effects on subcellular trafficking and aggregation of PMP22. The global modulators of protein raft affinity and raft modulators described here deepen our understanding of raft biophysics and present a new set of tools for probing the functional importance of lipid rafts and their constituent proteins.

POSTER #24

Unveiling ferrosome formation by cryo-electron tomography

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Recent research has identified ferrosome organelle as a potential iron storage mechanism in three Gram-negative environmental anaerobes. The exclusivity of ferrosome generation to Gram-negative bacteria, however, remains uncertain. Additionally, the fundamental processes involved in ferrosome formation are not well understood. In this study, we utilized cryo-electron tomography (cryo-ET) to discover ferrosomes in the Gram-positive human pathogen, *Clostridioides difficile*. These ferrosomes are encapsulated by lipid membranes and are frequently found adjacent to cellular membranes. We further demonstrated that the expression of FezA and FezB from *Clostridioides difficile* in *Escherichia coli*, which lacks an intracellular membrane system, leads to the formation of membrane vesicles. We discovered that these vesicles are equivalent in size to ferrosomes in *Clostridioides difficile* and are generated from the inner membranes of the cell. Notably, overexpression of FezA or FezB alone could not induce vesicle formation in *Escherichia coli*. Our findings suggest a novel mechanism for vesicle formation within the cell and offer a potential vehicle for drug delivery.

POSTER #25

Decoding the role of protein dynamics in heme biosynthesis initiation

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Heme is an essential biomolecule in processes such as oxygen transport and cellular differentiation. Biosynthesis is a multi-step route starting with the rate-limiting production of aminolevulinic acid (ALA), which is catalyzed by ALA synthase (ALAS). ALAS catalysis is controlled by the movement of an active site gate that is further regulated by homolog-specific mechanisms. My research uses structural, biochemical, and biophysical techniques to define the functional role of gate dynamics in yeast ALAS.

POSTER #26

Planning and Installing a Helium Liquefication Plant

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The world helium supply was finally supposed to have a major reprieve form a multi-year shortage in 2022, which resulted in restrictions of helium delivery. We all found out the hard way that this was not be the case. Several fires at the Russian state-owned production site of Gazprom in Amur, the impact by the Ukraine war, as well as unexpected maintenance issues at the BLM plant in Texas since July 2021 have severely impacted the supply chain in the last months again. This led to renewed helium shortages with allocations at the 60% levels. This cycle of shortages and allocations has been a re-occurring story for the last 15-20 years and prompted Vanderbilt University to invest into our own helium re-liquefying plant. While the concept is straight forward, specific information on many details was hard to come by when planning the liquefier plant. This poster describes our solution and provides many of the necessary detail when planning for, installing, and operation such a plant. We will discuss pressure issues, magnet hookup and safety considerations, choosing proper dimensions and their impacts, accessories that you might need, but are not necessarily provided or even discussed in the planning stage.

POSTER #27

Discovery of Small Molecule Modulators of PMP22 Expression and Trafficking in Rat Schwann Cells

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Myelin biogenesis requires tightly controlled trafficking of certain membrane proteins from the endoplasmic reticulum to the plasma membrane of Schwann cells. As such, interruption of membrane protein trafficking can have dire consequences for myelin formation and integrity. Mutations affecting the peripheral myelin protein 22 (PMP22) gene can decrease the protein's folding efficiency, thereby impairing its trafficking to the plasma membrane, subsequently resulting in Type 1 Charcot-Marie-Tooth disease (CMTD; CMT1). Duplications and deletions of the PMP22 gene result in moderate or mild neuropathy, respectively, suggesting an expression level dependence on folding, trafficking, and dysmyelination. Indeed, using a clonal population of Sprague-Dawley rat Schwann cells (RSCs) that express myc-tagged human PMP22 under the control of a tetracycline-inducible promoter (CMV-TetOn), we demonstrated that Myc-PMP22 folding and trafficking efficiency negatively correlate with its expression level in vitro. Correspondingly, immunofluorescence microscopy reveals that the average number of intracellularly retained Myc-PMP22 aggregates increases with higher expression. We therefore hypothesize that rectifying PMP22 expression, post-translational processing, and trafficking to its wild type levels is required to restore healthy myelination in patients with CMT1. No effective treatments targeting PMP22 expression or trafficking have been approved by the FDA. To address this lack of small molecule drugs, we have since completed a phenotypic high throughput screen in evaluating 22305 compounds for their effects on total levels of Myc-PMP22 at the plasma membrane in the inducible RSCs. Myc-PMP22 expression was induced simultaneously with compound treatment for 24 hours, after which the cells were fixed, immuno-stained for Myc-PMP22, and subsequently analyzed by high content immunofluorescence confocal microscopy. Of the compounds tested, 17 were demonstrated to reproducibly increase or decrease plasma membrane PMP22 levels. These compounds are being further characterized for their potency and toxicity, specificity toward Myc-PMP22 expression and trafficking, and effects on Myc-PMP22 folding and progression through its trafficking pathway. We hope to utilize these findings in further hit-to-lead drug discovery efforts, as well as to develop chemical probes of the PMP22 trafficking pathway.

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POSTER #28

Molecular mechanism of PIN1-mediated regulation of the nuclear receptor PPARγ

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Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand sensitive nuclear receptor and master regulator of adipogenesis. There exist FDA approved drugs that bind the ligand binding domain (LBD) of PPARγ to induce a well characterized conformational change, which alters the activity of this transcription factor. However, the functionally critical N-terminal intrinsically disordered AF1 domain remains poorly understood from a structural standpoint due to the limited number of biophysical methods that can provide atomic level detail of binding interactions and AF1 conformational states. Peptidyl-prolyl cis/trans isomerase NIMA-interacting 1 (PIN1) is a known protein binding partner of PPARγ that exerts its catalytic activity specifically at pSer/pThr-Pro motifs, facilitating the cis/trans isomerization of proline bonds along the peptide backbone. Preliminary data from our lab and others suggest PIN1 binds the AF1 region of PPARγ with a much greater affinity than the LBD, an observation which presents the opportunity to better understand the role of AF1 function in PPARγ biology. This binding interaction suggests a cascade of post translational modifications (PTMs), including kinase-mediated phosphorylation and PIN1 enzyme-catalyzed proline cist/trans isomerization of the AF1 region, may be responsible for tuning the transcriptional activity of PPARγ and maintaining activation of adipogenic gene programs. To structurally characterize phosphorylation-dependent and isomerization-dependent conformational changes within the AF-1 region, nuclear magnetic resonance (NMR) spectroscopy is extensively. To correlate the structural and molecular mechanisms described by NMR with functional effects observed in cells, this project relies on structure-guided mutagenesis, transcriptional reporter assays, and knockdown experiments in pre-adipocytes to understand the role of PIN1 as a mediator of PPARγ function during adipogenesis. Gene expression analysis and assays that measure cellular endpoints associated with PPARγ function will further assess the roles of these PTMs within the AF1 domain in promoting adipogenic function.

POSTER #29

Molecular basis of Nurr1-RXRα activation

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Small molecule compounds that activate transcription of Nurr1- RXRα (NR4A2-NR2B1) nuclear receptor heterodimers are implicated in the treatment of neurodegenerative disorders, but function through poorly understood mechanisms. Here, we show that RXRα ligands activate Nurr1-RXRα through a mechanism that involves ligand-binding domain (LBD) heterodimer protein- protein interaction (PPI) inhibition, a paradigm distinct from classical pharmacological mechanisms of ligand-dependent nuclear receptor modulation. NMR spectroscopy, protein-protein interaction, cellular transcription assays show that Nurr1-RXRα transcriptional activation by RXRα ligands is not correlated with classical RXRα agonism but instead correlated with weakening Nurr1-RXRα LBD heterodimer affinity and heterodimer dissociation. Our data inform a model by which pharmacologically distinct RXRα ligands (agonists and Nurr1-RXRα selective agonists that function as RXRα antagonists) operate as allosteric PPI inhibitors that release a transcriptionally active Nurr1 monomer from a repressive Nurr1-RXRα heterodimeric complex. These findings provide a molecular blueprint for ligand activation of Nurr1 transcription via small molecule targeting of Nurr1- RXRα.

POSTER #30

The CSB Green Team

The CSB Green Team is composed of graduate students, faculty, staff, and administrators across multiple departments at Vanderbilt University and Vanderbilt University Medical Center who all share a passion for the environment and making change. The goal of the CSB Green Team is to promote multi-level collaboration to create a more green-forward Vanderbilt and instill environmentally-conscious systems in biomedical research labs.

POSTER #31

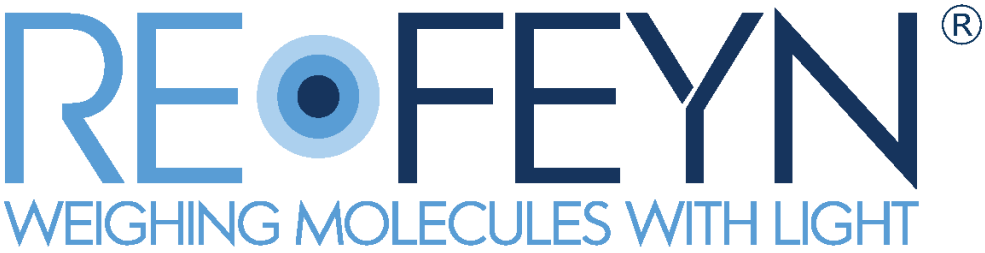
Small Angle X-ray Scattering (SAXS) as an assay tool for mRNA LNPs

Josue San Emeterio and Scott Barton

Xenocs Inc, Holyoke, MA

We demonstrate how Small Angle X-ray Scattering (SAXS) can be used as a molecular assay tool to identify pseudo-phases in mRNA LNPs (Lipid Nanoparticles) as a function of pH and temperature. The study was motivated by the use of SAXS to evaluate formulations during the development of Covid-19 vaccines and recent work that correlates scattering features observed in SAXS with therapeutic efficacy. The measurements were made on a laboratory SAXS instrument (Xenocs Xeuss 3.0) and do not require synchrotron radiation.

POSTER #32



Mass photometry – revolutionary biophysical characterization of single molecules

Kate Shields

Refeyn Inc., Watertown, MA

Mass photometry is a novel bioanalytical technology that provides single-molecule mass measurements of biomolecules in their native state within minutes without the need for labelling, surface immobilization or big sample quantities. Mass photometry is based on interferometric scattering microscopy, measuring single molecules thanks to an unprecedented level of sensitivity. Its ease of use makes mass photometry the perfect tool for rapid assessments of sample purity and homogeneity, structural integrity or macromolecular interactions across biomolecules ranging from differently sized proteins to DNA and even small viruses, such as AAVs. In this talk we will show how mass photometry can answer a wide range of questions in the structural biology and biochemistry fields and how it can be integrated with downstream structural biology approaches like CryoEM and crystallography.