

**CHEMICAL
BIOLOGY &
PHYSIOLOGY
2023**
PORTLAND, OR
DECEMBER 14-17

Program and Abstracts

December 14 – 17, 2023
Oregon Health & Science University
Portland, OR USA

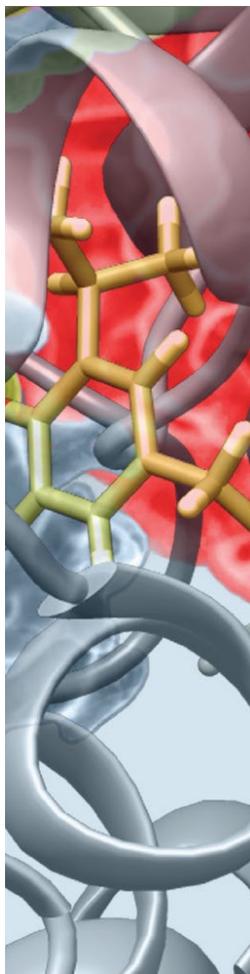


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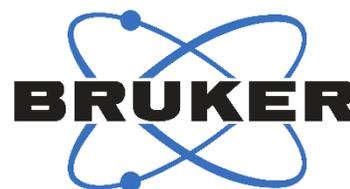
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SPECIAL THANKS TO:

Nick Crampton and Ken Welcome for AV support

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THURSDAY – DECEMBER 14

11:00 – 13:00	Check-in and Welcome
13:00 – 13:10	Opening Remarks

SESSION 1: CHEMICAL BIOANALYTICS

Chair: Elisar Barbar

13:10 – 13:45	Yimon Aye , Swiss Federal Institute of Technology Lausanne (EPFL) <i>What we do in the dark: Illuminating functional responsivity, signaling activity, & druggability of the local interactome in living systems</i>
13:45 – 14:00	Short Talk: Alix Thomas , Oregon Health & Science University <i>Development of FRET-based biosensors for measuring dynamic changes in ADP-ribosylation in cells</i>
14:00 – 14:35	Clifton Barry , NIH/NIAID <i>Exploiting a Bacterial Enzyme for Selective Prodrug Activation: Mycobacterium tuberculosis N-acetylates 5-aminomethyl oxazolidinones</i>
14:35 – 14:50	Short Talk: Xin Zhou , Dana-Farber Cancer Institute, Harvard Medical School <i>Targeted membrane protein degradation</i>
14:50 – 15:20	Break
15:20 – 15:35	Short Talk: Kaylin Fosnacht , University of Oregon <i>Thiol-Activated, Fluorogenic H₂S Donors for H₂S Delivery, Detection, and Application</i>
15:35 – 16:10	Neal Devaraj , University of California - San Diego <i>Precision Bioconjugation Chemistry Using Caged Tetrazines</i>
16:10 – 16:25	Short Talk: Sepehr Sebghati , University of Utah <i>Development of fluorogenic tools for studying antibiotic resistance in bacteria</i>
16:25 – 17:05	Flash Talks: Poster presenters
17:05 – 17:30	Break

KEYNOTE SPEAKER

Chair: Vickie DeRose

17:30 – 18:30	Squire Booker , The Pennsylvania State University and HHMI <i>A Radical Solution for C(sp³)-C(sp³) Bond Formation during the Biosynthesis of Macrocyclic Membrane Lipids</i>
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18:30 – 20:30	Reception
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FRIDAY – DECEMBER 15

SESSION 2: CHEMICAL PHYSIOLOGY

Chair: Sarahi Garza

09:00 – 09:35	Ehud Isacoff , UC Berkeley <i>Light-controlled neuromodulation</i>
09:35 – 10:10	Evan Miller , University of California - Berkeley <i>Fiat Lux! Using chemistry to visualize cellular physiology</i>

DAILY SCHEDULE

10:10 – 10:25	Short Talk: Meredith Hartley , University of Kansas <i>Lipid regulation during CNS myelin damage and repair</i>
10:25 – 10:50	Break – sponsored by ABBVIE
10:50 – 11:25	Mikhail Shapiro , California Institute of Technology, HHMI <i>Talking to cells: biomolecular ultrasound for deep tissue cellular imaging and control</i>
11:25 – 12:00	Emily Balskus , Harvard University, HHMI <i>Deciphering microbes and microbiomes with chemistry</i>
12:00 – 12:40	Flash Talks: Poster presenters
12:40 – 13:40	Lunch Meet the Speakers
13:40 – 15:10	Poster Session I – sponsored by AMGEN

SESSION 3: THE CHEMICAL BIOLOGY TOOLBOX

Chair: Kimberly Beatty

15:10 – 15:45	Jennifer Prescher , UC Irvine <i>Imaging tools and technologies inspired by nature's light-emitting chemistry</i>
15:45 – 16:20	Sara Buhrlage , Dana-Farber Cancer Institute, Harvard Medical School <i>Harnessing Deubiquitinases for Protein Stability Therapeutics</i>
16:20 – 16:35	Short Talk: Philipp Pöschko , Max Planck Institute for Medical Research Heidelberg <i>Light activatable plant-hormone based chemical inducers of proximity for in vivo applications</i>
16:35 – 17:10	Klaus Hahn , University of North Carolina - Chapel Hill <i>Watching and controlling the conformational changes of single molecules in living cells</i>
17:10 – 17:25	Break – sponsored by ABBVIE

KEYNOTE SPEAKER

Chair: Braden Lobingier

17:25 – 18:25	Alice Ting , Stanford University <i>Engineering proteins to map and manipulate cells</i>
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SATURDAY – DECEMBER 16

SESSION 4: NUCLEOTIDE CHEMICAL BIOLOGY

Chair: Daniel Bejan

09:00 – 09:35	John Lueck , University of Rochester School of Medicine and Dentistry <i>Development of Therapeutic Anticodon Edited (ACE)-tRNA Technologies for Nonsense Associated Diseases</i>
09:35 – 10:10	Nicolas Winssinger , University of Geneva <i>Using molecular assembly to program responsive biochemical systems</i>
10:10 – 10:25	Short Talk: Dennis Bong , The Ohio State University <i>URIL-tagging: a new chemical method for intracellular tracking and proximity-labeling of RNA-protein complexes</i>
10:25 – 10:55	Break – sponsored by GENENTECH
10:55 – 11:30	Amanda Hargrove , Duke University <i>Modulating the conformation and function of disease-relevant RNA with small molecules</i>

DAILY SCHEDULE

11:30 – 11:45	Short Talk: Jessica Simon , University of Washington <i>circRNA Barcoding: A versatile tool for performing high throughput biochemical profiling using next generation sequencing</i>
11:45 – 12:25	Flash Talks: Poster presenters
12:25 – 13:25	Lunch Meet the Editors
13:25 – 14:55	Poster Session II – sponsored by ACS CHEMICAL BIOLOGY

SESSION 5: PROTEIN CHEMISTRY

Chair: Ryan Mehl

14:55 – 15:30	Mark Howarth , University of Cambridge <i>Bacterial superglues, signal synergy and outbreak protection</i>
15:30 – 16:05	Kathrin Lang , ETH Zurich <i>Expanding the genetic code – new chemistries for biology</i>
16:05 – 16:20	Short Talk: Jason Zhang , University of Washington <i>De novo designed Hsp70 activator dissolves intracellular condensates</i>
16:20 – 16:55	Chang Liu , UC Irvine <i>Extensive gene evolution on laboratory timescales</i>
16:55 – 17:10	Break – sponsored by GENENTECH

KEYNOTE SPEAKER

Chair: Cat Vesely

17:10 – 18:10	Ronald Raines , Massachusetts Institute of Technology <i>Lessons from Collagen: Chemical Biology in the Extracellular Matrix</i>
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SUNDAY – DECEMBER 17

SESSION 6: STRUCTURAL CHEMICAL BIOLOGY

Chair: Liman Zhang

09:00 – 09:35	Andres Leschziner , University of California - San Diego <i>Structural biology of Parkinson's Disease-linked LRRK2</i>
09:35 – 10:10	Angela Gronenborn , University of Pittsburgh School of Medicine <i>The awesome power of fluorine NMR - from drugs to cells</i>
10:10 – 10:45	Meghna Gupta , University of California - San Francisco <i>Structural analysis of human Aquaporin 4 with Neuromyelitis Optica Spectrum Disorder patient derived pathogenic autoantibodies</i>
10:45 – 11:05	Break – sponsored by ROYAL SOCIETY OF CHEMISTRY
11:05 – 11:40	Michael Sattler , Helmholtz Munich and Technical University of Munich <i>Alternative splicing regulation by dynamic molecular recognition, RNA structure and small molecules</i>

KEYNOTE SPEAKER

Chair: Benjamin Barad

11:40 – 12:40	Eva Nogales , UC Berkeley <i>Structural Insights into the Regulation of the PRC2 Gene Silencer</i>
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12:40 – 12:55	Poster Prize Presentations
12:55 – 13:00	Closing Remarks
13:00	Lunch

WHAT WE DO IN THE DARK: ILLUMINATING FUNCTIONAL RESPONSIVITY, SIGNALING ACTIVITY, & DRUGGABILITY OF THE LOCAL INTERACTOME IN LIVING SYSTEMS

Yimon Aye – Swiss Federal Institute of Technology Lausanne (EPFL), Switzerland

I will highlight how an organic chemistry-driven idea has evolved into an enabling chemical biology toolset that addresses some of the long-standing as well as emerging key biomedical problems of importance in both fundamental and translational science. Emphasis will be placed on how deriving a quantitative understanding of precision electrophile signaling and pathway-level mechanistic studies open doors to new biological insights and functional drug discovery [1]. This research is currently supported by the grants from the European Research Council, Swiss National Science Foundation (PROJECT; SPIRIT; SPARK funding programs), Swiss Cancer League, and EPFL.

[1] Huang & Poganik et al., *Nat Protoc* **2023**, *18*, 1379. Zhao et al. *PNAS* **2022**, *119*, e2120687119. Poganik et al., *Nat Commun* **2021**, *5736*. Liu et al., *ACS Cent Sci* **2020**, *6*, 892. Long & Parvez et al., *Nat Chem Biol* **2017**, *13*, 333. Parvez & Long et al., *Nat Protoc* **2016**, *11*, 2328.

DECIPHERING MICROBES AND MICROBIOMES WITH CHEMISTRY

Emily Balskus, Ph.D. – Thomas Dudley Professor of Chemistry and Chemical Biology, Department of chemistry and Chemical Biology, Harvard University

The human body is colonized by trillions of microorganisms that exert a profound influence on human biology, in part by providing functional capabilities that extend beyond those of host cells. In particular, there is growing evidence linking chemical processes carried out by the human gut and vaginal microbiomes to various health outcomes ranging from cancer development to preterm birth. However, we still do not understand the vast majority of the molecular mechanisms underlying how the human microbiome influences host biological processes. Major obstacles faced in surmounting this knowledge gap include the difficulty linking functions associated with the human microbiome to specific microbial enzymes and the challenge of controlling these activities in complex microbial communities. This talk will discuss my lab's efforts to characterize gut and vaginal microbial metabolic activities that are linked to human health, understand their relevance in human microbiomes, and to develop tools and approaches to control microbial metabolism in complex microbial communities. Gaining a molecular understanding of microbial metabolic activities will not only help to elucidate the mechanisms by which these organisms affect host health but should also enable efforts to treat and prevent disease by manipulating our microbial communities.

EXPLOITING A BACTERIAL ENZYME FOR SELECTIVE PRODRUG ACTIVATION: MYCOBACTERIUM TUBERCULOSIS N-ACETYLATED 5-AMINOMETHYL OXAZOLIDINONES

Clifton Berry, Ph.D. – Senior Investigator, NIH/NIAID

Linezolid is a drug with proven human antitubercular activity whose use is limited to highly drug-resistant patients because of its toxicity. This toxicity is related to its mechanism of action – linezolid inhibits protein synthesis in both bacteria and eukaryotic mitochondria. A highly selective and potent series of oxazolidinones, bearing a 5-aminomethyl moiety (in place of the typical 5-acetamidomethyl moiety of linezolid) was identified. Linezolid resistant mutants were cross-resistant to these molecules, but not vice versa. Resistance to the 5-aminomethyl molecules mapped to an N-acetyl transferase (Rv0133) and these

mutants remained fully linezolid susceptible. Purified Rv0133 was shown to catalyze the transformation of the 5-aminomethyl oxazolidinones to their corresponding N-acetylated metabolites and this transformation was also observed in live cells of *Mycobacterium tuberculosis*. Mammalian mitochondria, which lack an appropriate N-acetyltransferase to activate these prodrugs, were not susceptible to inhibition with the 5-aminomethyl analogs. Several compounds that were more potent than linezolid were taken into C3HeB/FeJ mice and were shown to be highly efficacious and one of these (9) was additionally taken into marmosets and found to be highly active. Penetration of these 5-aminomethyl oxazolidinone prodrugs into caseum was excellent.

A RADICAL SOLUTION FOR C(sp³)-C(sp³) BOND FORMATION DURING THE BIOSYNTHESIS OF MACROCYCLIC MEMBRANE LIPIDS

Squire J. Booker, Ph.D. – Evan Pugh University Professor of Chemistry and of Biochemistry and Molecular Biology, The Pennsylvania State University and Howard Hughes Medical Institute

Archaea synthesize isoprenoid-based ether-linked membrane lipids, which enable them to withstand extreme environmental conditions, such as high temperatures, high salinity, and low or high pH values. In some archaea, such as *Methanocaldococcus jannaschii*, these lipids are further modified by forming carbon-carbon bonds between the termini of two lipid tails within one glycerophospholipid to generate the macrocyclic archaeol or forming two carbon-carbon bonds between the termini of two lipid tails from two glycerophospholipids to generate the macrocycle glycerol dibiphytanyl glycerol tetraether (GDGT). GDGT contains two 40-carbon lipid chains (biphytanyl chains) that span both leaflets of the membrane, providing enhanced stability to extreme conditions. How these specialized lipids are formed has puzzled scientists for decades. The reaction necessitates coupling two completely inert sp³-hybridized carbon centers, which has not been observed in nature. Here we use X-ray crystallography, high-resolution mass spectrometry, chemical synthesis, and biochemical analyses to show that the gene product of *mj0619* from *M. jannaschii*, which encodes a radical S-adenosylmethionine enzyme, is responsible for biphytanyl chain formation during synthesis of both the macrocyclic archaeol and GDGT membrane lipids.

HARNESSING DEUBIQUITINASES FOR PROTEIN STABILITY THERAPEUTICS

Sara Buhrlage, Ph.D. - Department of Cancer Biology, Dana-Farber Cancer Institute and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

DUBs, through cleavage of the isopeptide bond linking ubiquitin and ubiquitin-like proteins to substrates, play a key role in proteolysis as well as localization, interactome and activation of diverse proteins. Increasingly, links between DUBs and disease are being discovered, with perhaps the most excitement around the promise of precision degradation of pathogenic proteins via DUB inhibition. Concomitant with appreciation of the role of DUBs in disease, is a need for high quality chemical probes to study DUB function and pharmacologically validate members of the enzyme family as disease targets. My lab has established a platform for discovery, optimization and rigorous characterization of DUB inhibitors. Our platform integrates DUB library synthesis, medicinal chemistry, biochemistry, high-throughput screening, chemoproteomics, chemical genomics, structural biology, target validation and cancer biology. I will show how the platform has enabled rapid access to hits, leads and probes for DUBs, and present our work using first-in-class probes to study DUB biology in both biased and unbiased fashions.

PRECISION BIOCONJUGATION CHEMISTRY USING CAGED TETRAZINES

Neal Devaraj, Ph.D. – Department of Chemistry and Biochemistry, University of California, San Diego

Our lab is broadly interested in leveraging advances in chemical biology and systems chemistry to mimic and study the remarkable properties of lipid membranes. Inspired by our ongoing work on developing lipid bioconjugation strategies to generate artificial cell membranes, we have also developed new tools for manipulating biomolecules. I will discuss progress on developing selective bioconjugation chemistries that are suitable for use within living cells. Specifically, we have recently become interested in developing methods to control the bioconjugation of lipids and other molecules in a stimuli responsive manner, for instance after exposure to a light source. While there has been a recent surge in the available chemistries that are compatible in living cells, achieving cell or subcellular specificity remains challenging. I will discuss recent efforts in developing chemically “caged” tetrazines as stimuli responsive bioconjugation agents that can be used for precision chemistry in living cells. In addition to their application in tagging biomolecules such as lipids, I will also discuss how caged tetrazines may be useful for specifically controlling the synthesis and release of therapeutics.

THE AWESOME POWER OF FLUORINE NMR - FROM DRUGS TO CELLS

Angela M. Gronenborn, Ph.D. - Department of Structural Biology, School of Medicine, Department of Bioengineering, Swanson School of Engineering, Department of Chemistry, Dietrich School of Arts and Sciences, University of Pittsburgh

Nuclear magnetic resonance (NMR) spectroscopy is a versatile tool for probing structure, dynamics, folding, and interactions at atomic resolution. While naturally occurring magnetically active isotopes, such as ^1H , ^{13}C , or ^{15}N , are most commonly used in biomolecular NMR, with ^{15}N and ^{13}C isotopic labeling routinely employed at the present time, ^{19}F is a very attractive and sensitive alternative nucleus, which offers rich information on biomolecules in solution and in the solid state. This presentation will summarize the unique benefits of solution, solid-state and in-cell ^{19}F NMR spectroscopy for the study of biomolecular systems. Particular focus will be placed on the most recent studies and on unique and important potential applications of fluorine NMR methodology.

STRUCTURAL ANALYSIS OF HUMAN AQUAPORIN 4 WITH NEUROMYELITIS OPTICA SPECTRUM DISORDER PATIENT DERIVED PATHOGENIC AUTOANTIBODIES

Meghna Gupta¹, Nitesh Khandelwal¹, Andrew Nelson¹, Sergei Pourmal¹, Jeffery Bennett², Robert M. Stroud¹

¹University of California San Francisco, San Francisco, CA, USA

²University of Colorado, Anschutz Medical Campus, Anschutz, CO, USA

Neuromyelitis Optica Spectrum Disorder (NMOSD) is an autoimmune disease where human antibodies self-target the water channel AQP4. Pathogenic AQP4 autoantibodies (AQP4-IgG) bind to AQP4 on astrocytes of the central nervous system. This leads to activation of the complement cascade ultimately resulting in astrocyte death, then death of oligodendrocytes, resulting in demyelination and neuronal loss. Eventually, tissue injury and neurological impairment happens through both lytic and non-lytic mechanisms. NMOSD patients have a panel of circulating autoantibodies, and the mechanism of immune response varies from antibody to antibody. We determined molecular structures of AQP4 apo and AQP4

bound to patient derived autoantibodies in lipid nanodiscs using cryo-EM. These are the first structures of a channel demonstrating an autoimmune disease. Our data provides insights into high-resolution aspects of the interface between AQP4 and autoantibodies for drug designing. Using these structures, we highlight the binding pattern differences among antibodies and possible correlation with the immune responses generated at the cellular level. These outcomes can be used to develop a better drug-development platform.

WATCHING AND CONTROLLING THE CONFORMATIONAL CHANGES OF SINGLE MOLECULES IN LIVING CELLS

Klaus Hahn, Ph.D. – Thurman Distinguished Professor of Pharmacology, University of North Carolina - Chapel Hill

Control of cell behavior relies on the subtle kinetics and localization of protein conformational changes. This talk will focus on new live cell approaches to shed light on these events, including causality analysis based on multiplexed imaging of biosensors and optogenetics, stretch-induced exposure of binding sites on individual molecules, and engineered domains that can be inserted into proteins for control by light or small molecules. These approaches will be applied to probe how cancer cells migrate from tumors to the vasculature via aligned collagen fibers.

MODULATING THE CONFORMATION AND FUNCTION OF DISEASE-RELEVANT RNA WITH SMALL MOLECULES

Prof. Amanda E. Hargrove, Ph.D. – Duke University, Durham, North Carolina, USA

Small molecules offer a unique opportunity to target structural and regulatory elements in therapeutically relevant RNAs, but understanding functional selectivity has been a recurrent challenge in small molecule:RNA recognition. RNAs tend to be more dynamic and offer less chemical functionality than proteins, and biologically active ligands must compete with the highly abundant and highly structured RNA of the ribosome. Indeed, the first and only small molecule drug targeting RNA other than the ribosome was approved by the US FDA in August of 2020. Our recent survey of the literature revealed less than two hundred reported chemical probes that target non-ribosomal RNA in biological systems.

As part of our efforts to improve small molecule targeting strategies and gain fundamental insights into small molecule:RNA recognition, we have analyzed patterns in both RNA-biased small molecule chemical space and RNA topological space privileged for differentiation. We have applied these principles to functionally modulate conformations of 3'-triple helix of the long noncoding RNA MALAT1, leading to small molecule degraders, as well as in the development of RNA-targeted antivirals for enterovirus (EV71) and SARS-CoV-2.

BACTERIAL SUPERGLUES, SIGNAL SYNERGY AND OUTBREAK PROTECTION

Mark Howarth, Ph.D. – Department of Pharmacology, Cambridge University, UK

The bacterium *Streptococcus pyogenes* has evolved spontaneous isopeptide bond formation within various surface proteins. We have re-engineered this protein chemistry to generate an irreversible peptide-protein interaction (SpyTag/SpyCatcher). This reaction is genetically-encodable and shows

specificity in diverse biological environments. We accelerated reactivity towards the diffusion limit and prepared a toolbox for rapidly controlling protein architectures. With reversible SpyTag interaction tuned by pH or temperature, we established efficient protein purification through the SpySwitch system. SpyMask inducible reactivity allowed simple assembly of spatially controlled bispecific antibody panels, to change the output of receptor signaling in cancer cells. We also developed an independent bacterial superglue called NeissLock, enabling covalent reaction to unmodified human proteins via anhydride formation. I will discuss applications to cell therapy and vaccination, including for broad protection against emerging pandemic threats.

LIGHT-CONTROLLED NEUROMODULATION

Ehud Isacoff, Ph.D. – Professor, Dept of Molecular & Cell Biology, University of California, Berkeley

Dopamine regulates motor function, motivation, learning, reward, aversion, attention, sleep and appetite. These functions are mediated by dopaminergic projections that spread broadly in the brain, which activate five different dopamine receptors (DRs) with distinct signaling properties and downstream effectors. These receptors may be found in multiple cell types, which are inter-connected in complex patterns, and a cell may express more than one DR subtype and the receptors can be located in different places in the cell. Moreover, the timing of dopamine signaling is often critical to neural computations. I will describe a method for dynamic control of specific dopamine receptors that can work in select brain areas, cell types and subcellular compartments.

EXPANDING THE GENETIC CODE – NEW CHEMISTRIES FOR BIOLOGY

Kathrin Lang, Ph.D. – Professor for Chemical Biology, Department of Chemistry and Applied Biosciences (D-CHAB), ETH Zurich

Nature uses a limited set of twenty amino acids to synthesize proteins. In recent years it has become possible to site-specifically incorporate designer amino acids with tailored chemical properties into proteins in living cells by reprogramming the genetic code. Together with developments in designing chemical reactions that are applicable to and selective within living systems, these strategies have begun to have a direct impact on studying biological processes.

In this talk I will present our lab's efforts to expand the genetic code and to endow proteins with novel chemical moieties within their physiological environment. By site-specifically incorporating artificial designer amino acids into proteins, we have developed tools to image and probe proteins,^[1,2] to study protein-protein interactions and stabilize low-affinity protein complexes^[3-6] and to re-engineer and manipulate molecular networks and biological pathways such as ubiquitylation in living cells.^[7-9]

We envision that these approaches and technologies will enable the study of biological processes that are difficult or impossible to address by more classical methods.

[1] K. Lang et al.; *Chem. Rev.* 2014, 114, 4764.

[2] S.V. Mayer et al.; *Angew. Chem. Int. Ed.* 2019, 58, 15876.

[3] M. Cigler et al.; *Angew. Chem. Int. Ed.* 2017, 56, 15737.

[4] T.A. Nguyen et al.; *Angew. Chem. Int. Ed.* 2018, 57, 14350.

[5] T.A. Nguyen et al.; *Angew. Chem. Int. Ed.* 2022, 61, e20211108.

[6] J. Du et al., *Nat. Commun.* 2021, 12, 460, doi: 10.1038/s41467-020-20702-2.

[7] M. Fottner et al.; *Nat. Chem. Biol.* 2019, 15, 276.

[8] M. Fottner et al.; Nat. Commun. 2021, 12, 6515.

[9] M. Fottner et al.; J. Am. Chem. Soc. 2022, 144, 13118.

STRUCTURAL BIOLOGY OF PARKINSON'S DISEASE-LINKED LRRK2

Andres Leschziner, Ph.D. – Professor, Department of Cellular and Molecular Medicine, School of Medicine and Section of Molecular Biology, Division of Biological Sciences, University of California - San Diego

Parkinson's Disease (PD) is the second most prevalent neurodegenerative disease, affecting ~10 million people worldwide. One of the most commonly mutated genes in PD codes for **Leucine Rich Repeat Kinase 2 (LRRK2)**. Autosomal dominant mutations in *LRRK2* cause familial PD, while mutations in *LRRK2* are risk factors for sporadic PD and increased activity of LRRK2's kinase has been linked to the sporadic form of the disease as well. LRRK2 is a large protein with multiple domains, including both a kinase and a GTPase. A unifying theme for *LRRK2* and many other PD genes is that they function in intracellular trafficking. For example, LRRK2 co-localizes with microtubules, an association that is enhanced by some PD mutations and has been shown to disrupt trafficking in model systems.

As part of a multi-lab team, we are combining structural biology (cryo-EM and cryo-ET), single-molecule biophysics, chemistry, biochemistry, and cell biology, to understand the role of LRRK2 in cells, and how PD-linked mutations affect its function. We are also interested in understanding the potential side effects of small-molecule therapeutics that target LRRK2 to treat PD.

EXTENSIVE GENE EVOLUTION ON LABORATORY TIMESCALES

Chang Liu, Ph.D. - Professor and Chancellor's Fellow of Biomedical Engineering, Chemistry, and Molecular Biology & Biochemistry, Director, Center for Synthetic Biology, University of California, Irvine

What does the map between macromolecular sequence and function look like? How are nearly infinite high dimensional sequence spaces, such as those defining RNA and protein function, productively searched? How does a gene's evolutionary past shape its future? To answer these questions, my group engineers genetic systems that dramatically accelerate the speed of gene evolution so that we can prospectively watch and systematically manipulate the course of long gene evolutionary processes on laboratory timescales. I will share a recent upgrading of our orthogonal DNA replication system (OrthoRep) to enable the experimental evolution of chosen genes at 1-million times the genomic error rate *in vivo*. This allowed us to extensively adapt and diverge an enzyme in a way that generates enormous diversity, from which we were able to detect new selective forces shaping how the gene evolved *in vivo*. I will also share new work on the application of OrthoRep to antibody and enzyme engineering.

DEVELOPMENT OF THERAPEUTIC ANTICODON EDITED (ACE)-TRNA TECHNOLOGIES FOR NONSENSE ASSOCIATED DISEASES

John Lueck, Ph.D. – Department of Pharmacology and Physiology, University of Rochester School of Medicine and Dentistry

Nonsense mutations are single-nucleotide mutations that convert a canonical amino acid codon to one of the three stop codons (UAA, UAG, and UGA). Nonsense mutations introduce a premature termination codon (PTC) generally with almost complete loss of function of the affected protein both due to translation of a truncated protein product and loss of mRNA transcript triggered by nonsense mediated

decay (NMD). With growing access to human genomic sequence data, more than 7,500 nonsense mutations in nearly 1,000 different human genes have been discovered. PTCs account for close to 11% of all described protein variants leading to inherited human disease. Known PTC-associated disease phenotypes include Duchenne muscular dystrophy, inherited retinal disorders, Hurler syndrome, β -thalassemia, Dravet syndrome and cystic fibrosis. We are developing technologies based on anticodon edited (ACE) tRNAs, for readthrough of disease-causing PTCs, restoration of transcript abundance and therapeutic meaningful levels of active protein. Focused on nonsense-associated CF, we have demonstrated that delivery of ACE-tRNAs results in significant rescue of endogenous cystic fibrosis transmembrane conductance regulator (CFTR) channel function in immortalized airway and primary human intestinal epithelial cells. Important for developing a platform CF therapeutic, we found that one ACE-tRNA isotype sequence results in significant rescue of endogenous CFTR function expressed from alleles with four different, but the most prevalent, CF nonsense variants. We are now focusing on overcoming the airway epithelial cell delivery hurdle through modifications to the ACE-tRNA therapeutic cargo and pairing with emerging viral and nonviral delivery vectors.

FIAT LUX! USING CHEMISTRY TO VISUALIZE CELLULAR PHYSIOLOGY

Evan Miller, Ph.D. – University of California, Berkeley

Cells spend a substantial portion of their energy budget to maintain an electrochemical potential difference across the plasma membrane. Optical methods to measure changes in cellular membrane potential provide a powerful complement to traditional, electrode-based methods by providing spatial resolution. My lab has been developing synthetic, voltage-sensitive fluorescent indicators that respond to changes in membrane potential via a photoinduced electron transfer mechanism. In this presentation, I will discuss my group's recent efforts to apply fluorescent indicators to living cells to interrogate cellular physiology.

STRUCTURAL INSIGHTS INTO THE REGULATION OF THE PRC2 GENE SILENCER

Eva Nogales, Ph.D. – Howard Hughes Medical Institute investigator; a Professor of Biochemistry and Molecular Biology at the University of California, Berkeley; and Senior Faculty Scientist at the Lawrence Berkeley National Laboratory

Polycomb repressive complex 2 (PRC2) is an epigenetic regulator responsible for the trimethylation of histone H3 at lysine 27. This chromatin mark leads to the silencing of key genes, an essential process for both embryonic development and the maintenance of cell identity. PRC2 function is regulated through several mechanisms, including its association with various accessory proteins and its recognition of different histone posttranslational modifications. We have used cryo-EM to shed light into the different molecular mechanisms that regulate the activity of PRC2, including how the complex can be recruited by PRC1-deposited H2A119Ub, or how its activity is stimulated by its own H3K27me3 product, leading to spread of the mark. This spreading is thought to be limited by the inhibition of PRC2 activity on nucleosomes containing H3K36me3 and H3K4me3 modifications, which localize to sites of active transcription, by a currently unknown mechanism. I will show how our cryo-EM studies reveal that the inhibition involves changes in distinct structural elements within PRC2 or at the PRC2-nucleosome interface. Our work also supports a key role for the JARID2 cofactor in modulating PRC2 activity in the presence of different histone modifications. Additionally, EZH2, the PRC2 subunit with methyltransferase activity, has been shown recently to be automethylated *in cis*, leading to increased enzymatic activity by a

mechanism that is still unclear. We have studied the structural implications of substrate chromatin engagement and methylation in the absence of any allosteric activator other than automethylated EZH2 to show that, in a novel chromatin- and automethylation-dependent manner, PRC2 dimerizes such that an automethylated inactive PRC2 complex serves as an allosteric activator of a second substrate-engaged PRC2. We propose that dimerization-dependent stimulation of PRC2 activity in *trans*, driven by EZH2 automethylation and local PRC2 concentration, represents a new mechanism to regulate the establishment of H3K27me3 heterochromatin domains.

IMAGING TOOLS AND TECHNOLOGIES INSPIRED BY NATURE'S LIGHT-EMITTING CHEMISTRY

Jennifer Prescher, Ph.D. – Professor, University of California, Irvine

Imaging tools enable researchers to spy on cells and biomolecules and track their behaviors in real time. While powerful, many of these probes are limited to studies on microscope slides or in culture dishes. Visualizing biological processes in more authentic environments—heterogeneous tissues and whole animals—requires tools that can function over longer time and length scales. Toward this end, my group is developing general probes for imaging cellular and molecular features *in vivo*. Our work leverages bioluminescence, nature's light-emitting chemistry with luciferase enzymes and luciferin small molecules. Bioluminescence can circumvent historical challenges with imaging in tissues and organisms, and permit sensitive tracking over time. This talk will showcase our efforts to develop engineered luciferases and luciferins for noninvasive visualization, along with novel detection platforms. Applications of the technologies in biological imaging will also be highlighted. Collectively, these pursuits are enhancing our understanding of living systems and enabling new discoveries.

LESSONS FROM COLLAGEN: CHEMICAL BIOLOGY IN THE EXTRACELLULAR MATRIX

Ronald T. Raines, Ph.D. – Massachusetts Institute of Technology

Collagen is the most abundant protein in animals, including humans. A typical human body has 10 pounds of collagen in its extracellular matrix. Dinosaurs also deployed collagen as their bodily scaffold. Collagen strands wind into a tight triple helix. Each strand contains many 4-hydroxyproline (Hyp) residues, resulting from the most prevalent post-translational modification in animals.

Using synthetic collagen-mimetic peptides (CMPs) that contain 4-fluoroproline and other subtly non-natural residues, we have shown that previously unappreciated forces—stereoelectronic effects—are responsible for the increased stability endowed upon the collagen triple helix by its prevalent Hyp residues. This discovery led us to articulate the importance of C=O...C=O *n-to-π** interactions between main-chain carbonyl groups as a significant contributor to the conformational stability of not only collagen but all proteins. Exploiting these stereoelectronic effects with synthetic amino acids has enabled us to exert exquisite control over collagen stability. Especially promising are CMPs that anneal specifically to the damaged collagen triple helices in wound beds, fibrotic tissue, and the tumor microenvironment tumors. This annealing can anchor pendant probes or chemotherapeutic agents at the site of collagen damage *in vivo*, providing new modalities for the clinical detection and treatment of wounds, fibrosis, cancer, and other indications. The approach is akin to antibody–drug conjugates but with much simpler molecules and mechanisms of action.



***n*→*π** Interaction**

ALTERNATIVE SPLICING REGULATION BY DYNAMIC MOLECULAR RECOGNITION, RNA STRUCTURE AND SMALL MOLECULES

Michael Sattler, Ph.D. – Institute of Structural Biology, Molecular Targets & Therapeutics Center, Helmholtz Munich, Neuherberg, Germany, Bavarian NMR Center, Department of Bioscience, TUM School of Natural Sciences, Technical University of Munich, Garching, Germany

Gene regulation at the level of RNA is controlled by dynamic protein-RNA interactions, RNA structure and posttranscriptional modifications. We employ integrative structural biology, combining solution NMR, small angle scattering, crystallography and cryo-EM to study the structure and conformational dynamics of multidomain RNA-binding proteins and RNAs involved in these processes. Our studies highlight how structure and dynamics of proteins and RNAs control their biological function. Examples will be provided with protein-RNA and protein-protein interactions in the regulation of alternative pre-mRNA splicing, and on-going research towards the modulation of these interactions using small molecules.

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TALKING TO CELLS: BIOMOLECULAR ULTRASOUND FOR DEEP TISSUE CELLULAR IMAGING AND CONTROL

Mikhail G. Shapiro, Ph.D. – Max Delbrück Professor of Chemical Engineering and Medical Engineering, California Institute of Technology, Investigator, Howard Hughes Medical Institute

The study of biological function in intact organisms and the development of targeted cellular therapeutics necessitate methods to image and control cellular function *in vivo*. Technologies such as fluorescent proteins and optogenetics serve this purpose in small, translucent specimens, but are limited by the poor penetration of light into deeper tissues. In contrast, most non-invasive techniques such as ultrasound and magnetic resonance imaging – while based on energy forms that penetrate tissue effectively – are not effectively coupled to cellular function. Our work attempts to bridge this gap by engineering biomolecules with the appropriate physical properties to interact with magnetic fields and sound waves. In this talk, I will describe our recent development of biomolecular reporters and actuators for ultrasound. The reporters are based on gas vesicles – a unique class of gas-filled protein nanostructures from buoyant photosynthetic microbes. These proteins produce nonlinear scattering of sound waves, enabling their detection with ultrasound. I will describe our recent progress in understanding the biophysical and acoustic properties of these biomolecules, engineering their mechanics and targeting at the genetic level, developing methods to enhance their detection *in vivo*, expressing them heterologously as reporter genes, and turning them into dynamic sensors of intracellular molecular signals. In addition to their applications in imaging, gas vesicles can be used to control cellular location and function by serving as receivers of acoustic radiation force or seeding localized bubble cavitation. Additional remote control is provided by thermal bioswitches – biomolecules that provide switch-like control of gene expression in response to small changes in temperature. This allows us to use focused ultrasound to remote-control engineered cells *in vivo*.

ENGINEERING PROTEINS TO MAP AND MANIPULATE CELLS

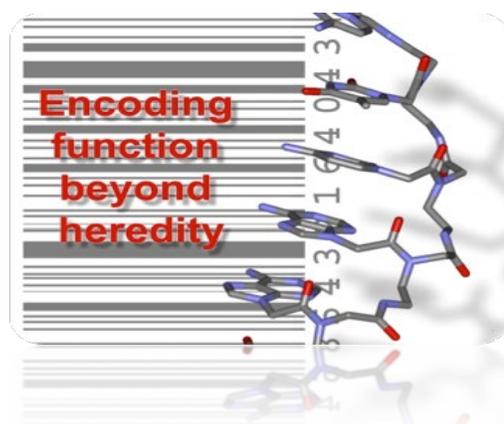
Alice Y. Ting, Ph.D. – Departments of Genetics, Biology, and by courtesy, Chemistry, Stanford University

Biological function is orchestrated through the collective action of molecules and cells. Our laboratory develops technologies to study molecular and cellular assemblies at high spatial and temporal resolution in living cultures and organisms. I will describe recent technologies from our laboratory including TransitID for unbiased mapping of proteome trafficking within cells and between cells, light-regulated proximity labeling, and new enzymes for proximity labeling created through machine learning. I will also describe new technologies for manipulating cellular behavior in response to specific external cues, through the use of engineered synthetic receptors.

USING MOLECULAR ASSEMBLY TO PROGRAM RESPONSIVE BIOCHEMICAL SYSTEMS

Nicolas Winssinger, Ph.D. – Organic Chemistry Department, University of Geneva

Life is orchestrated by biomolecules interacting in complex networks of biological circuitry with emerging function. Progress in different areas of chemistry has made the design of systems that can recapitulate elements of such circuitry possible. At the circuitry level, the programmable nature of nucleic acid hybridization provides a powerful platform to design dynamic systems that can respond and integrate diverse logic gates. In order to interface with diverse biomolecular inputs (e.g. cell surface receptors or therapeutic target) and yield outputs other than oligonucleotide sequences (e.g. drugs or fluorophores for sensing) it is desirable to engineer nucleic acid conjugate that can translate assemblies into output through proximity enhanced reactions. Peptide Nucleic Acids (PNA) are endowed with attractive properties for this endeavor as they are more robust and form more stable duplex than their natural counter parts. Several applications from our laboratory to encode and program self-assemblies of small molecules, template chemical reactions and respond to biomarkers will be presented.



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#30 - URIL-TAGGING: A NEW CHEMICAL METHOD FOR INTRACELLULAR TRACKING AND PROXIMITY-LABELING OF RNA-PROTEIN COMPLEXES

Dr. Yufeng Liang, The Ohio State University

Sydney Willey, The Ohio State University

Dr. Yu-Chieh Chung, The Ohio State University

Dr. Shiqin Miao, The Ohio State University

Yi-Meng Lo, The Ohio State University

Prof Li-Chun Tu, The Ohio State University

Prof Dennis Bong, The Ohio State University

Internal, genetically encoded, uridine-rich internal loops (URILs) comprised of four contiguous UU pairs (8 nt) in RNA may be targeted with minimal structural perturbation by triplex hybridization with cell-permeable bifacial Peptide Nucleic Acids (bPNAs) that are ~1kD molecular weight. This URIL-tagging strategy for RNA and DNA tracking has a light molecular footprint and minimizes structural alterations to the RNA of interest. Here we fluorogenic URIL-tagging of endogenous RNAs and RNPs in fixed and live cells (HEK293T, U2OS) that operates in conjunction with the gold standard in RNA labeling (bacteriophage MS2). A series of lentivirus modified cell lines were generated that express U1snRNAs that are URIL tagged in each of the 4 U1snRNA stem loops. We report herein our preliminary data in intracellular fluorogenic RNA tracking of these constructs, as well as intracellular proximity biotinylation studies using photoredox active bPNA probes. Overall, URIL tagging is a versatile chemical approach for RNP tracking and proximity labeling that has unique advantages in the investigation of RNA interactome biology, target identification and development of new therapeutics.

#25 - THIOL-ACTIVATED, FLUOROGENIC H₂S DONORS FOR H₂S DELIVERY, DETECTION, AND APPLICATION

Kaylin Fosnacht, University of Oregon

Prof. Michael Pluth, University of Oregon

Although hydrogen sulfide (H₂S) was originally known as a highly toxic gas made through processes such as volcanic activity or modern sewage systems, more recently the association of H₂S with cellular processes and function has been uncovered. Decreases in H₂S have been shown to be a potential biomarker of Parkinson's disease, asthma, and Huntington's disease, and H₂S releasing compounds have been developed as one approach to mediate the severity of such diseases. One limitation of most H₂S donors is that the release rates in vitro often differ from those in more complex environments. One approach to solve this challenge is to develop H₂S donors that provide an optical response upon H₂S delivery. Although H₂S donors with a fluorescent response have been reported previously, a palette of H₂S donors across different wavelengths that use the same H₂S-releasing approach has remained absent. Herein, we report five thiol-activated, sulfenyl thiocarbonate-based COS/H₂S releasing donors that employ blue, yellow, orange, red, and near-infrared emitting dyes to produce a fluorescence turn-on response. Upon reaction of a thiol with the sulfenyl thiocarbonate scaffold, a disulfide and the deprotected dye are released along with COS. In the presence of the mammalian enzyme carbonic anhydrase, COS is quickly converted into H₂S. All donors resulted in a fluorescence turn-on (3–310-fold) and concurrent H₂S release (>60% efficiency). All of the developed donors were confirmed to release H₂S in live cells, which was confirmed by the use of additional H₂S-responsive probes. In addition, the NIR donor was applied to live rats to successfully observe a fluorescence turn-on over time.

#20 - LIPID REGULATION DURING CNS MYELIN DAMAGE AND REPAIR

Ms. Nishama de Silva Mohotti, University of Kansas

Ms. Hiroko Kobayashi, University of Kansas

Ms. Jenna Williams, University of Kansas

Mr. Matthew Zupan, University of Kansas

Ms. Esther Holt, University of Kansas

Ms. Rashmi Binjawadagi, University of Kansas

Mr. Ziyu Zhu, University of Kansas

Dr. Meredith Hartley, University of Kansas

Myelin damage is a major feature of multiple sclerosis and is also observed in other neurological diseases including Alzheimer's, however, there are no FDA-approved therapies that directly target myelin repair. Formation of the lipid-rich myelin sheath is induced by hormones, and our goal is to define how CNS lipids are regulated during neurological diseases with demyelination. We have used genetic mouse models of demyelination to profile lipid dynamics during disease. Brain, spinal cord, and plasma samples from distinct demyelinating and remyelinating phases were analyzed by mass spectrometry and the levels of over 300 distinct lipids were measured. Principal component analysis was used to demonstrate that the CNS lipidome has unique signatures during demyelination and remyelination. Volcano plot analysis was used to identify lipid classes and lipid species that differentially regulated during demyelination and remyelination. The analysis also revealed that remyelination in the spinal cord is greatly impaired relative to the brain. Our current research is focused on several areas. (1) Cholesterol esters were identified in the lipidomics panel as potential CNS biomarkers of myelin damage. We are currently performing experiments to identify which enzymes and proteins are involved in the regulation of cholesterol esters. Our goal is to better understand how cholesterol ester formation impacts the ability of the CNS to successfully remyelinate. (2) We are interested in exploring how different hormones (thyroid hormone and estrogen) impact lipid regulation during demyelination to better understand how hormones regulate CNS lipids during disease.

#34 - LIGHT ACTIVATABLE PLANT-HORMONE BASED CHEMICAL INDUCERS OF PROXIMITY FOR IN VIVO APPLICATIONS

Philipp Pöschko, Max Planck Institute for Medical Research, Heidelberg, Germany

Caroline Berrou, Max Planck Institute for Medical Research, Heidelberg, Germany

Kaisa Pakari, Centre for Organismal Studies, Heidelberg University, Heidelberg, Germany

Michael Ziegler, Max Planck Institute for Medical Research, Heidelberg, Germany

Birgit Koch, Max Planck Institute for Medical Research, Heidelberg, Germany

Kai Johnsson, Max Planck Institute for Medical Research, Heidelberg, Germany

Joachim Wittbrodt, Centre for Organismal Studies, Heidelberg University, Heidelberg, Germany

Richard Wombacher, Max Planck Institute for Medical Research, Heidelberg, Germany

Interactions between proteins are substantial for the regulation of cellular mechanisms and methods to control these processes are highly desirable. Chemical inducers of proximity (CIPs) are an attractive tool to precisely manipulate protein-protein interaction in live cells and in vivo. Here, we present pMandi, a photoactivatable derivative of the plant hormone-based CIP mandipropamid (Mandi) enabling to use light as an external control to induce protein proximity in mammalian cells. We further demonstrate that the abscisic acid agonist opabactin (OP) can be used as CIP in mammalian cells at low nanomolar concentrations. Its photocaged derivative pOP enables the use of light to induce protein proximity in single cells improving spatiotemporal control up to single cell resolution. Additionally, we investigate the application of both CIPs and their photocaged derivatives to induce protein proximity in live medaka embryos.

#78 - DEVELOPMENT OF FLUOROGENIC TOOLS FOR STUDYING ANTIBIOTIC RESISTANCE IN BACTERIA

Mr. Sepehr Sebghati, University of Utah

Prof. Ming Chen Hammond, University of Utah

Antibiotic resistance is a major global health challenge and a growing public health concern. It is estimated that by the year 2050, antimicrobial resistance could lead to 10 million deaths annually worldwide ¹. Some of the most common mechanisms of resistance formation in bacteria include a decrease in the amount of antibiotic uptake, either through a decrease in the membrane permeability of the drug or through the overexpression of efflux pumps, mutation of antibiotic's target in a manner that prevents engagement of the drug with its target, and the inactivation of the antibiotic molecule by bacterial enzymes. Due to the increase in the prevalence of antibiotic-resistant bacterial strains, methods that allow for studying the mechanisms of resistance formation in bacteria are urgently needed. Recently we established a high-throughput assay to assess antibiotic permeability in Gram-positive and negative bacteria. This assay uses antibiotic-conjugated fluorogenic dyes that bind to a specific RNA aptamer inside the cytoplasm of bacterial cells. Upon binding of the fluorogenic antibiotic probe to the RNA aptamer, fluorescence is activated and enhanced. The resulting fluorescence correlates with the relative permeability of the antibiotic and is then measured using flow cytometry or fluorescence microscopy. Here we aim to expand this work by investigating the ability of these fluorogenic antibiotic probes to study mechanisms of resistance formation such as efflux pump activity and target mutation. Preliminary data suggests that these fluorogenic antibiotics are versatile tools that can report on the activity of efflux pumps and target engagement. We expect these probes would provide a rapid, real-time, high throughput live-cell assay that could have widespread applications including facilitating the discovery of

efflux pump inhibitors, the rapid identification of resistant strains in clinical samples and the identification of the mode of action of promising antibiotic molecules.

#59 - CIRC RNA BARCODING: A VERSATILE TOOL FOR PERFORMING HIGH THROUGHPUT BIOCHEMICAL PROFILING USING NEXT GENERATION SEQUENCING

Jessica Simon, University of Washington

Prof. Douglas Fowler, University of Washington

Prof. Dustin Maly, University of Washington

The ability to measure the functional properties of thousands of protein variants using high throughput sequencing can provide fundamental insight into protein function, the pathogenesis of disease-associated mutations, and drug resistance and sensitivity. However, current methods for protein variant profiling are indirect and do not allow for the biochemical measurement of many pooled protein variants in a mammalian intracellular environment. To address this gap, we developed a novel biochemical profiling method, circRNA barcoding, which combines the strengths of proteomics with the sensitivity, throughput, and the ability to quantify protein variants of Next Generation Sequencing. This method uses the assembly of ribonucleoprotein complexes within mammalian cells, directly tethering protein variants with hyper-stable RNA barcodes. Subsequent enrichment of protein variants from lysate co-enriches associated barcodes, facilitating protein quantification using Next Generation Sequencing. We show how this technology can be used to quantify protein abundance, activity, and protein-protein interactions for thousands of pooled variants in tandem. We demonstrate how our method was used to elucidate the biochemical characteristics of 1,600 variants of the oncogene B-Raf at 80 clinically-relevant positions. Our analysis provides key insights into how B-Raf mutations affect protein folding, ability to drive downstream ERK signaling, interactions with

intracellular binding partners (C-Raf and MEK), and sensitivity to clinically-relevant inhibitors. Collectively, these experiments showcase the versatility of circRNA barcoding and underscore its potential in facilitating comprehensive analyses across the human proteome.

#35 - DEVELOPMENT OF FRET-BASED BIOSENSORS FOR MEASURING DYNAMIC CHANGES IN ADP-RIBOSYLATION IN CELLS

Alix Thomas, Oregon Health & Science University

Daniel Bejan, Oregon Health & Science University

Prof. Michael Cohen, Oregon Health & Science University

Prof. Carsten Schultz, Oregon Health & Science University

ADP-ribosylation, the addition of ADP-ribose (ADPR) groups, is a highly conserved chemical modification occurring in cells resulting in dynamic and diverse signaling cascades. This process is regulated by writers, the PARPs enzyme, erasers and readers. Two of those writers, PARP1 and PARP2, are early responders of DNA damage in human cells and important anti-cancer drug targets. These use NAD(+) to modify proteins with mono or poly ADP-ribose leading to chromatin decompaction and recruitment of DNA repair factor. Although antibodies and mass spec have allowed the specific detection of ADPR, they can fail to capture the dynamic turn-over of ADP-ribosylation. We interrogated whether it would be possible to quantify ADP-ribosylation in real time using genetically encoded fluorescent probes. For that we took advantage of characterized mono and poly ADPR recognition sequences and a known substrate for PARP1 upon DNA damage to design a series of FRET probes. We have shown that these probes are capable of detecting specifically poly and mono ADPR in living cells in time and space after DNA damage. We believe that these tools will broaden the tool box of ADPR sensors and will offer for the first time a spatially and temporally resolved mono-ADPR sensor.

#29 - DE NOVO DESIGNED Hsp70 ACTIVATOR DISSOLVES INTRACELLULAR CONDENSATES

Dr. Jason Zhang, University of Washington

Nathan Greenwood, University of Washington

Jason Hernandez, University of California San Francisco

Professor Josh T Cuperus, University of Washington

Dr. Buwei Huang, University of Washington

Dr. Bryan D Ryder, University of California San Francisco

Professor Christine Queitsch, University of Washington

Professor Jason E Gestwicki, University of California San Francisco

Professor David Baker, University of Washington

Protein quality control (PQC) is carried out in part by the chaperone Hsp70, in concert with adapters of the J-domain protein (JDP) family. The JDPs, also called Hsp40s, are thought to recruit Hsp70 into complexes with specific client proteins. However, the molecular principles regulating this process are not well understood. We describe the de novo design of a set of Hsp70 binding proteins that either inhibited or stimulated Hsp70's ATPase activity; a stimulating design promoted the refolding of denatured luciferase in vitro, similar to native JDPs. Targeting of this design to intracellular condensates resulted in their nearly complete dissolution. The designs inform our understanding of chaperone structure-function relationships and provide a general and modular way to target PQC systems to condensates and other cellular targets.

#4 - TARGETED MEMBRANE PROTEIN DEGRADATION

Dr. Dingpeng Zhang, Dana-Farber Cancer Institute, Harvard Medical School

Ms. Jhoely Duque-Jimenez, Dana-Farber Cancer Institute, Harvard Medical School

Mr. Garyk Brixi, Harvard University

Dr. Francesco Facchinetti, Dana-Farber Cancer Institute, Harvard Medical School

Ms. Kaitlin Rhee, Dana-Farber Cancer Institute, Harvard Medical School

Dr. William W. Feng, Dana-Farber Cancer Institute, Harvard Medical School

Prof. Pasi A. Jänne, Dana-Farber Cancer Institute, Harvard Medical School

Prof. Xin Zhou, Dana-Farber Cancer Institute, Harvard Medical School

<https://zhou-lab.org/>

Membrane proteins, such as receptor tyrosine kinases, checkpoint receptors, and G-protein coupled receptors, play vital roles in cell signaling and mediating interactions between cells and their environment. The development of a universal strategy to selectively degrade endogenous membrane proteins would have significant value in both basic research and therapeutic interventions. We have recently developed a versatile and modular membrane protein degradation technology utilizing a novel family of hetero-bispecific antibodies. This approach leverages the rapid endocytosis of a widely distributed endogenous receptor found in human cells. During our presentation, we will describe our protein engineering strategies employed in the design of degraders for diverse membrane proteins. Furthermore, we will show the applications of this technology in reversibly controlling Chimeric Antigen Receptor (CAR)-T cell activities and in targeting drug-resistant epidermal growth factor receptor (EGFR) mutant lung cancers. This technology presents a promising avenue for modulating cell surface protein levels and activities, opening new opportunities for targeted cell modulation in research and medicine.

#60 - HIGH-RESOLUTION IMAGING OF NUCLEOLAR PROCESSES INFLUENCED BY METAL COMPOUNDS

Katelyn R. Alley, University of Oregon

Katelyn M. Wyatt, University of Oregon

Prof. Victoria J. DeRose, University of Oregon

Platinum anticancer compounds are in worldwide clinical use. In cells, the labile ligands of Pt(II) chemotherapeutics undergo exchange with biological targets, forming stable Pt(II) adducts. While Pt(II) compounds share common characteristics such as the ability to bind to DNA, recently oxaliplatin has been established to act through a unique pathway in the nucleolus. The nucleolus is a critical membraneless nuclear organelle that serves as the site of ribosome synthesis. Oxaliplatin acts through the inhibition of nucleolar processes with subsequent cell death. Given the importance of nucleolar targeting by Pt(II) chemotherapeutics, creating robust tools for measuring nucleolar function is necessary to better understand the mechanisms of action of compounds that cause nucleolar stress. One limitation in the field has been insufficient resolution in standard fluorescence imaging studies that are needed for understanding precise locations and interactions of nucleolar proteins and their changes under stress-causing conditions. To overcome this barrier, we are employing technology that allows high to near super-resolution imaging using a classic confocal microscope. Expansion microscopy enables super-resolution imaging of biological samples by physical enlargement through isotropic polymerization networks. In expansion microscopy, fluorescently-tagged biomolecules are crosslinked to swellable hydrogels, which are then isotropically expanded. Post-expansion, the tagged biomolecule shows an increase in effective resolution, improving localization and quantification when compared to conventional imaging techniques. We applied this methodology to investigate the nanoscale organization of nucleolar proteins. To provide

further insight into the molecular mechanism of Pt(II) compounds this methodology is being coupled with click chemistry to monitor the localization of the metal compounds and the effects of the compounds on rRNA synthesis. Tracking small metal complexes and investigating their impact on the nucleolus at very high resolution will provide new insight into their localization and impact on an essential cellular process and may open a new window for therapeutic targeting.

#88 - DEVELOPING A CHEMICALLY-CONTROLLED RAS-TOOLSET

Fernando Banales Mejia, University of Washington

Dr. Dustin J. Maly, University of Washington

This study investigates the nuanced spatiotemporal regulation of RAS, a crucial protein toggling between "on/off" states in cellular signaling. Understanding this compartmentalization provides a clearer roadmap for innovative RAS-targeting strategies. Two key areas are explored. The first focuses on the essential membrane localization for RAS activation, using an EGFR-based fusion with NS3a-CIAR, a chemically inducible protein switch triggering wild-type endogenous RAS. Specifically, this study dissects the specific extracellular elements of EGFR crucial for RAS activation. The second segment probes how local cellular conditions influence the kinetics and intensity of RAS activation. Here, an engineered EGFR-based NS3a-CIAR serves as a reporter, illuminating the impact of the cellular environment on RAS activation dynamics. Insights from this research promise a deeper comprehension of how RAS signaling is intricately regulated in space and time. This understanding may open new avenues for precise RAS modulation, potentially offering novel therapeutic strategies for RAS-mediated disorders.

#54 - TOWARDS AN UNTARGETED, CONTINUOUS EVOLUTION PLATFORM FOR THE OPTIMIZATION OF GENETIC CODE EXPANSION MACHINERY

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Richard B. Cooley, Oregon State University

Ryan A. Mehl, Oregon State University

A major unmet challenge in genetic code expansion technology is the inherently low activity of encoding machinery, which are often ~3 orders of magnitude less catalytically efficient than their natural counterparts. These undesirable efficiencies often arise due to the nature of current selection approaches, which rely on saturation mutagenesis of only a few residues, severely limiting both the breadth and depth of mutational space that can be sampled. An untargeted selection approach that is not limited by combinatorial library sizes, on the other hand, would be unfettered in mutational space and would grant access to novel evolutionary trajectories that are currently inaccessible. Here, we describe our progress towards this goal by developing a Continuous Adaptive Laboratory Evolution platform for Genetic code expansion Optimization (CALEGO). This system relies on the addition and encoding of noncanonical amino acids for the biosynthesis of a critical metabolite under auxotrophic positive selection conditions, and the withholding of noncanonical amino acids and subsequent truncation of the same enzyme(s) in the presence of an antimetabolic prodrug under negative selection conditions. Concurrently, the number of TAG sites is increased over time to apply a selective pressure that links improvements in encoding efficiency to cellular fitness while simultaneously excluding escape mutants. Improving the efficiency of genetic code expansion machinery promises to grant access to new materials such as encodable noncanonical polymers, enhance the industrial potential of genetic code expansion, and improve the ease-of-use, applicability, and utility of

genetic code expansion tools to a greater community of researchers.

#40 - MIXED ALKYL/ARYL PHOSPHONATES DETERMINES MULTIPLE PATHWAYS FOR LIPID METABOLISM INHIBITORS AND A MECHANISM OF MULTIDRUG RESISTANCE IN PLASMODIUM FALCIPARUM

Mr. John Bennett, Stanford University

Dr. Sunil Narwal, Columbia University

Ms. Stephanie Kabeche, Stanford University

Dr. Daniel Abegg, University of Illinois Chicago

Dr. Fiona Hackett, Crick Institute

Ms. Veronica Li, Stanford University,

Mr. Franco Faucher, Stanford University

Mr. Ryan Muir, Stanford University

Prof. Michael Blackman, Crick Institute

Prof. Alex Adibekian, University of Illinois Chicago

Prof. David Fidock, Columbia University

Prof. Matthew Bogyo, Stanford University

Resistance to frontline treatments against Plasmodium falciparum severely threatens public health and highlights the need for new therapeutic strategies. We previously demonstrated that the potent antimalarial natural product Salinipostin A (SalA) kills parasites with an overall low propensity to generate resistance. The molecule functions by disrupting lipid metabolism through the inhibition of multiple serine hydrolase targets. Given the inherent difficulty in using complex natural products as therapeutic agents, we synthesized a small library of simple mixed alkyl/aryl phosphonates containing lipid chains as bioisosteres of SalA. We identified a pair of constitutional isomers with dramatically different overall anti-parasite killing potencies. Using a combination of chemoproteomic, genetic, and phenotypic approaches we determined the active isomer kills parasites through a mechanism that is distinct from both SalA and the pan-lipase inhibitor, Orlistat. Importantly, we found that, like SalA, this new class of anti-parasite agents

has multiple targets which results in only weak resistance generation that can be attributed to mutations in the same PRELI domain-containing protein identified in parasites with weak resistance to SalA. Together, these data suggest that mixed alkyl/aryl phosphonates are a synthetically tractable new class of anti-malaria agents with a distinct mechanism of action that has an overall low propensity to induce resistance.

#42 - TARGETING AND MODULATING POSITIVE-SENSE VIRAL RNA REGULATORY STRUCTURES WITH AMILORIDE-BASED SMALL MOLECULES

TinTin Bui Luu, Duke University

Kanika Chopra, Transylvania University

Dr. Martina Zafferani, Duke University

Dr. Shinya Suzuki, Duke University

Dr. Amanda E. Hargrove, Duke University

The rapid and widespread occurrence of viral diseases, such as the recent SARS-CoV-2 pandemic and enterovirus 71 (EV71) epidemic, raises urgency to design new antivirals and investigate novel targets to combat emerging variants and future viral outbreaks. SARS-CoV-2 and EV71 contain highly conserved secondary structure stem loops (SLs) within the 5'untranslated region (UTR) that are essential for viral translation and replication. Of particular significance, the SLII domain of EV71 interacts with RNA-binding proteins to modulate translation levels, and SL1-6 of SARS-CoV-2 are functionally critical for the selective promotion of viral transcript translation and replication over host cell mRNA and protein synthesis. Thus, non-coding, positive-sense viral RNA structures offer unique and attractive opportunities to identify novel therapeutic targets and to modulate their structures and regulatory functions with small molecule antivirals. This work builds upon an existing RNA-biased library of amiloride-based small molecules with several lead amilorides that have demonstrated dose-dependent inhibition of

viral translation and replication with no significant toxicity in cellular studies. Our previous synthetic strategies focused on C5 and C6 modifications, thus we have developed a strategy to increase the chemical diversity of amilorides by continuing to functionalize the C5 and C6 positions and have established a synthetic route to explore the newly accessible C3 position of the core scaffold. Expansion of this library and further exploring amiloride recognition of positive-sense viral RNA secondary structures will reveal fundamental insights into functional selectivity and improve potency of our lead antivirals. We carried out screening of this amiloride library to identify binders to several SLs necessary for EV71 and SARS-CoV-2 translation and have gained preliminary trends into the molecular properties of amilorides critical for their affinity. Currently in parallel, we are screening the library in a dual-luciferase functional assay that directly reports the small molecule effects on viral translation of the 5'ends of EV71 and SARS-CoV-2 in cellulo. Collectively, this work will elucidate structure-activity relationships of amiloride-based small molecules built on their binding versus inhibition of viral translation against these viral RNA secondary structures. Ultimately, we will define guiding principles for targeting and modulating viral RNA structures with small molecules and provide lead RNA-targeted antivirals for treatment and to further our understanding of viral gene expression and replication mechanisms.

#92 - STREAMLINING CHEMOPROTEOMICS WITH FULLY FUNCTIONALIZED ISOBARIC TAGS

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Mass spectrometry-based chemoproteomics has emerged as an enabling technology for functional biology and drug discovery. To address limitations of established chemoproteomics workflows, including cumbersome reagent synthesis and low throughput sample preparation, here, we established the silane-based cleavable isotopically labeled proteomics (sCIP) method. The sCIP method is enabled by a high yielding and scalable route to dialkoxydiphenylsilane fluorenylmethyloxycarbonyl (DADPS-Fmoc)-protected amino acid building blocks, which enable the facile synthesis of customizable, isotopically labeled, and chemically cleavable biotin capture reagents. sCIP is compatible with both MS1- and MS2-based quantitation, and the sCIP-MS2 method is distinguished by its click-assembled isobaric tags in which the reporter group is encoded in the sCIP capture reagent and balancer in the pan cysteine-reactive probe. The sCIP-MS2 workflow streamlines sample preparation with early-stage isobaric labeling and sample pooling, allowing for high coverage and increased sample throughput via customized low cost six-plex sample multiplexing. We further expanded our technology to be compatible with commercially available tandem mass tag (TMT) reagents resulting in a 1.5-fold decrease in sample preparation time compared to traditional TMT cysteine profiling workflows.

#12 - TLC-MALDI-TOF AS A PLATFORM FOR INVESTIGATING POLY-ADP-RIBOSE POLYMERASE ACTIVITY AND SPECIFICITY

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 Mr. Sean R. Wallace, Santa Clara University
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Poly (ADP-ribose) polymerases, PARPs, are a family of 17 enzymes (in humans) that were identified based on their ability to transfer ADP-ribose onto target proteins from nicotinamide adenine dinucleotide (NAD⁺). The ADP-ribosylation reaction occurs in a highly conserved PARP catalytic domain found in all fifteen of the active PARPs. Significant effort using modern proteomic methods has led to the identification of thousands of potential PARP targets in the cell. This analysis has not yet uncovered which sites are preferentially targeted *in vivo*, nor the determinants for PARP family-member specific targeting. Further, current mass spectrometric analytical methods require proteolysis of the target protein, limiting the study of dynamic ADP-ribosylation. Herein, we present a matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) method using thin-layer chromatography (TLC) that facilitates population-wide analysis of ADP-ribosylation. Using this method, we identify, for the first time, a minimal peptide fragment (5 amino-acids) that is preferentially modified by PARP14. We have also identified the specific glutamate (E) residue that is targeted on this peptide. Using this method, we observe divergent ADP-ribosylation dynamics for the catalytic domains of PARPs 14 and 15, with PARP15 modifying more sites on itself (+3-4 ADP-ribose) than the closely related PARP14 protein (+1-2 ADP-ribose) - despite similar numbers of potential modification sites. We further demonstrate through mutagenesis and chemical treatment with hydroxylamine that PARPs 14 and 15 prefer acidic residues. We measure the stability of the resultant ester bond

and show that non-enzymatic removal is sequence independent and occurs within hours. Finally, we use the ADPr-peptide to highlight differential activities within the glycohydrolase family and their sequence specificities. Our results highlight: 1) the utility of MALDI-TOF in motif discovery and 2) the importance of peptide sequence in governing ADPr transfer and removal.

#90 - COMPUTATIONAL DESIGN OF LIPOID, AN ULTRAFAST AND VERSATILE PROXIMITY LABELING ENZYME

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Enzyme-catalyzed protein proximity labeling (PL) has enabled the spatial- and temporal- characterization of proteomes in living systems. However, current PL enzymes are limited by long labeling times, requirement for toxic reagents, or restriction to biotin-based probes. Here we used protein language model guided evolution and structure-based computational protein design methods to engineer Lipoid, a promiscuous mutant of *E. coli* lipoic acid ligase A. Computationally predicted designs were screened via a mammalian FACS-based assay for enzymatic activity and stability, resulting in the identification of LplA mutants with orders of magnitude improvement in labeling activity. Lipoid has efficient activity with a range of substrates, including a variety of clickable handles such as azide, trans-cyclooctene, or

bicyclononyne substrates, and enables robust labeling within 1 minute, combining the rapidity of peroxidase-based proximity labeling with the non-toxicity of biotin-ligases. We have demonstrated the spatial specificity and sensitivity of Lipoid labeling in cell culture and are now applying the tool in vivo. We are exploring the broad potential applications of this new tool, including ELISA-based readout of protein-protein interactions and in-vivo profiling of protein translocation. Our results show that Lipoid is a powerful tool for efficient, precise, and non-toxic proximity labeling of proteomes in complex biological systems.

#38 - DYNAMICS OF A GLUTAMATE TRANSPORTER ORTHOLOG

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Glutamate transporters also referred to as excitatory amino acid transporters (EAATs) use ionic gradients across cell membranes for the concentrative uptake of glutamate. Our present understanding of the mechanism of glutamate uptake in EAATs is mainly from studies on the archaeal ortholog Glt_{Ph} which is a Na⁺-coupled aspartate transporter. A critical aspect of the transport cycle is the coupled binding of Na⁺ and aspartate. Previous studies have elucidated a major role for hairpin 2 (HP2), which functions as the extracellular gate for the aspartate binding site. These studies propose that opening of the HP2 with Na⁺ and the closing of HP2 with aspartate underlies the coupled binding observed. However, the exact mechanism by which Na⁺ mediates HP2 opening and aspartate binding mediates HP2 closure is not understood. To probe these mechanisms, we have developed an assay to monitor, in real-time, the movement of HP2 by the strategic incorporation of tryptophan and the unnatural amino acid, p-cyanophenylalanine (PheCN). By using this assay along with extensive mutagenesis of Glt_{Ph}, we have identified the residues and the specific

interactions that are critical for Na⁺ and aspartate-dependent HP2 movement. Based on our data and previous studies, we can provide the mechanism that underlies Na⁺ and aspartate dependent HP2 opening and closing. We anticipate that the strategy used in this study will be useful to probe short range protein dynamics in other proteins.

#94 - RECONSTITUTION OF REVERSIBLE GTPASE ACTIVATION USING LIGHT INDUCED SIGNALING INPUTS

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The ability of cells to transiently alter the concentration and spatial distribution of signaling molecules is a hallmark of cellular organization and signal adaptation. At the plasma membrane, spatial heterogeneity in cell signaling emerges from biochemical reactions involving phosphatidylinositol phosphate (PIP) lipids, PIP kinases, PIP phosphatases, and Rho-family GTPases. Interconnected positive and negative feedback loops are thought to control the communication between these distinct families of signaling molecules to create emergent properties, such as polarization, cortical oscillations, and transient spikes in activity. Although genetics and cell biology approaches have identified several classes of molecules that regulate these emergent properties, we do not currently know enough about these systems for biochemical reconstitution using a bottom-up approach. Here, we describe a new in vitro system to reconstitute minimal signal adaptation modules that are built around the communication between small GTPases and PIP lipid modifying enzymes. To this end, we established an optogenetics based system that utilizes the iLID-SspB light-induced protein heterodimerization systems to spatial and

temporal control the activation of small GTPase's and PIP lipid phosphorylation on supported membranes in vitro. We use this approach to dissect the communicate between Ras GTPase and phosphatidylinositol 3-kinase (PI3K).

#2 - ENDOSOMAL SORTING OF THE MU OPIOID RECEPTOR

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Opioids are both highly effective pain medications and the cause of exponentially increasing overdose deaths. Fully characterizing the mechanism behind their physiological effects is critical for advancements in both palliative and addiction medicine. On a cellular level, opioids exert their effects by binding to the mu-opioid receptor (MOR), a G protein-coupled receptor (GPCR). GPCR signaling is classically regulated through beta-arrestin binding to the receptor and inducing internalization into the cell. The GPCRs are then trafficked to the endosome and then the lysosome for degradation. However, some GPCRs, including MORs, can be rescued from a degradative fate and returned to the plasma membrane via recycling from the endosome. Importantly, the MOR's ability to recycle affects the development of opioid tolerance. Thus, an in-depth understanding of opioid tolerance will include knowledge of the mechanism by which MOR recycling occurs. MOR recycling is known to be dependent on a specific sequence of amino acids - "LENL" - in the receptor's C-terminal tail. However, identifying the cellular machinery that recognizes and acts upon this recycling sequence has been an insurmountable challenge due to the transient and low affinity nature of these interactions, and to this date the mechanism remains unknown. Recent technological advances have finally made

it possible to overcome this obstacle. Proximity labeling screens studying MOR trafficking identified the protein complex Retromer near MOR when the receptor was located on endosomes. Genetic screens of two receptors containing the MOR recycling motif also identified Retromer as a positive regulator of MOR recycling. Depletion of Retromer from HEK293 cells stably expressing MOR decreased receptor recycling and increased degradation. Overall, these findings suggest that Retromer plays a central role in the mechanism by which MOR recycles.

#62 - ACTIVITY-BASED PROBES FOR ASSESSMENT OF B-LACTAM ANTIBIOTIC TARGETS IN MYCOBACTERIAL PATHOGENS

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Mycobacterium tuberculosis (Mtb) is the bacterial pathogen that causes Tuberculosis (TB), the deadliest infectious disease in human history. In 2021 alone, over 10 million new TB infections arose globally and 1.6 million people died of TB¹. Mycobacterial infections are difficult to treat and cure, requiring months of treatment with multiple antibiotics. β -lactam antibiotics are some of safest, cheapest, and most accessible antibiotics, but are only considered for treating certain forms TB. This is partially because *Mtb* encodes the active β -lactamase BlaC. However, there is evidence that β -lactams are

therapeutically-relevant drugs for TB². Firstly, BlaC activity is readily inhibited by clinically-approved β -lactamase inhibitors, such as clavulanate³. Combining clavulanate with a β -lactam antibiotic showed potent inhibition of drug-resistant patient isolates *in vitro*⁴. Additionally, there are recent reports of successful treatment of TB patients via co-administration of clavulanate with β -lactam antibiotics^{5,6}. The combination of meropenem-amoxicillin/clavulanate led to an 83% cure rate in patients with extensively-drug resistant TB⁶. A better understanding of which *Mtb* enzymes are inhibited by specific β -lactams would provide additional information to support their use against TB. Our team has undertaken these studies using activity-based probes to identify and characterize the enzyme targets of β -lactam antibiotics. In our 2021 paper, we labeled *Mtb* lysates with meropenem-Cy5 and found that certain β -lactam antibiotics inhibited enzymes that make cell wall peptidoglycans⁷. Targets of meropenem included D,D-transpeptidases, L,D-transpeptidases, carboxypeptidases, and BlaC. We are now using a new probe suitable for selective protein enrichment, meropenem-biotin, to identify and compare β -lactam targets in mycobacteria grown in standard, dormancy-inducing, and lipid-rich conditions.

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- (7) Levine, S. R.; Beatty, K. E. *ACS Infect. Dis.* **2021**, *7*, 461-470.

#50 - IDENTIFICATION OF A NEW KATP CHANNEL MODULATOR FROM CRYO-EM STRUCTURE BASED VIRTUAL SCREENING

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Congenital hyperinsulinism (CHI) is a neonatal disease characterized by persistent insulin secretion despite life-threatening hypoglycemia. It can cause irreversible brain damage if not promptly treated. The most common genetic cause of CHI is the loss-of function mutations in *ABCC8* and *KCNJ11* which are the genes encoding for sulfonylurea receptor 1 (SUR1) and inwardly rectifier K⁺ channel (Kir6.2) proteins. These protein subunits assemble to compose the hetero-octameric complex of the ATP sensitive K⁺ (K_{ATP}) channels, with four central K⁺ conducting Kir6.2 pore subunits surrounded by four regulatory SUR1 subunits. K_{ATP} channels reside in the plasma membrane (PM) of pancreatic β -cells and act as a primary regulator of insulin secretion by adjusting PM potential in response to varying blood glucose levels. Loss-of function mutations can lead to either gating defects, or PM expression defects due to improper channel folding, assembly, or trafficking. The consequent reduction in K_{ATP} currents in both cases leads to a state of persistent β -cell membrane depolarization and therefore uncontrolled insulin secretion. Most CHI patients with K_{ATP} trafficking defects are unresponsive to K⁺ channel opener diazoxide and require pancreatectomy.

Pharmacochaperones (PCs), which are small molecules that bind to their target proteins to facilitate biogenesis and correct misfolding, hold great promise as a novel therapeutic avenue for CHI caused by K_{ATP} trafficking mutations. Our group previously reported several high affinity K_{ATP} inhibitors that act as efficient PCs to correct K_{ATP} trafficking defects, however, the clinical use of these drugs is hampered by their high affinity binding, which inhibits the function of the rescued channels. Recent cryo-EM structures of K_{ATP} channels bound to inhibitors including repaglinide provide an opportunity for structure-based drug discovery in the treatment of CHI.

AtomNet® technology, a deep convolutional neural network for structure-based drug discovery developed by Atomwise, was used to perform a virtual screen against a repaglinide-bound channel structure. A subset of the top-scoring compounds was subjected to biochemical and functional assays, testing their ability to rescue surface expression and function of K_{ATP} trafficking mutants. A novel compound that exhibits robust chaperoning effects on K_{ATP} channel trafficking mutations and inhibits normal K_{ATP} channel current in a reversible manner was identified. This PC compound showed specificity to pancreatic K_{ATP} channels over other K_{ATP} channels isoforms. The identification of new pancreatic K_{ATP} specific PCs that can rescue channel trafficking without compromising channel function may provide potential novel pharmacological treatments for patients suffering from CHI caused by K_{ATP} trafficking defects.

#16 - STRUCTURAL INSIGHTS INTO THE INHIBITION OF PHENYLALANYL-TRNA SYNTHETASE FROM MYCOBACTERIUM TUBERCULOSIS PROVIDES OPPORTUNITY FOR ANTI-TB DRUG DEVELOPMENT

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Bacterial aminoacyl-tRNA synthetases (aaRS), essential enzymes in protein synthesis, have recently emerged as promising drug targets. They play a critical role in translating the genetic code by attaching specific amino acids to their cognate tRNA molecules, offering multiple druggable binding sites (e.g., amino acid, ATP, tRNA, and editing). Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), remains a

deadly infectious disease with multidrug-resistant strains posing a severe public health threat, necessitating innovative treatments. In this study, we present crystal structures of the *Mtb* PheRS/tRNA^{Phe} complex bound with fragments, identified through Nuclear Magnetic Resonance (NMR) and Surface Plasmon Resonance (SPR) screenings. We validated the binding of these compounds via biochemical assays, demonstrating their competitive inhibition against phenylalanine. These findings offer insights for the rational design of anti-TB drugs. Our research represents a significant stride towards combatting TB, addressing the urgent need for new therapeutic approaches against this formidable infectious disease.

#80 - PLATINUM COMPOUNDS AND NUCLEOLAR STRESS: INFLUENCE OF OXIDATION STATE AND ELECTRONIC TUNING

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Small molecule platinum compounds, most notably cisplatin, oxaliplatin, and carboplatin, have been extensively studied for their biological roles as anti-cancer therapeutics. The mechanism of action of these compounds was believed to work through the DNA damage response (DDR) pathway. Recent work has shown, however, that oxaliplatin is unique and instead works through a nucleolar stress pathway. Nucleolar stress occurs when there are disruptions to the cellular nucleolus, a subsection of the nucleus where ribosome biogenesis occurs. Studies have found that properties of the non-labile ligands of anti-cancer platinum compounds influence their ability to cause nucleolar stress. Here, two properties are explored: electronic tuning and Pt oxidation state.

The nucleolar stress response is sensitive to changes in non-labile ligand orientation, steric-bulk, and hydrophobicity. However, current work has not yet identified the role of electronics in this process. Electronic properties are hypothesized to be a tunable factor that influences the reactivity of platinum complexes in cells. Specifically, electron donating groups on the o-phenylene diamine (OPDA) ligand may result in a faster aquation rate, leading to increased biological effects due to more rapid binding to biomolecules. To investigate this, a library of (OPDA)PtCl₂ variants will be made with a range of substituents that vary in degree of electron-withdrawing to donating nature. The ability and rate of complex binding to DNA will be assessed through gel electrophoresis. Nucleolar stress induction will be assessed through an NPM1 redistribution assay in A549 cells. Bond lengths and ligand pKa's will be investigated through single crystal XRD, UV-Vis absorption spectroscopy, and IR spectroscopy. Cellular uptake will be evaluated through inductively coupled plasma mass spectrometry (ICP-MS).

Pt(IV) compounds have been investigated as alternatives to Pt(II) compounds for their added functionality, stemming from two additional axial ligands. When activated in cells, Pt(IV) compounds are reduced, releasing the parent Pt(II) compound and corresponding axial ligands. These axial ligands can be used to grant favorable properties by acting as targeting agents, bioactive moieties, or as innocent spectators. By increasing the lipophilicity of the parent Pt(II) drug through short-chain fatty acid axial ligands, Pt(II) compounds that usually act through DDR may also induce nucleolar stress, presumably due to increased cellular uptake. Cellular platinum accumulation of the short-chain fatty acid Pt(IV) compounds is being determined by ICP-MS and cytotoxicity will be determined with the MTT assay. Additionally, induction of DDR is being assessed by activation of γH2AX in A549 cell lines, and the induction of nucleolar stress is being monitored as mentioned

previously. Future studies will focus on the influence organelle targeting axial ligands have on the mechanism of action of platinum compounds.

#18 - ENDOGENOUSLY EXPRESSION OF KINETOPLASTID EDITOSOME PROTEIN COMPLEXES FOR STRUCTURAL INVESTIGATION

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Prof. Liman Zhang, Oregon Health & Science University

RNA editing in kinetoplastids, like *Trypanosoma brucei*, the causing agent for trypanosomiasis, and *Leishmania donovani*, the causing agent for Leishmaniasis, is processed by a series of protein complexes. These complexes, referred to collectively as “editosome”, are composed of RNA-editing catalytic complex, and RNA-editing substrate-binding complex (RESC). Capturing the structural characteristics of these complexes is crucial to understanding the mechanism and process. Utilizing the *Leishmania tarentolae* expression system, we can target specific subunits in the editosome complexes in the non-pathogenic kinetoplast species and endogenously express them on a large scale. We constructed affinity-tagged cell lines to purify RECC and validated target protein expression via mass spectrometry. With cryo-electron microscopy, this expression system can help us further understand the structure and function of the complexes.

#32 - REGULATION OF TUMOR SUPPRESSOR 53BP1 OLIGOMERIZATION BY THE HUB PROTEIN LC8

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Genomic instability is a feature of various cancers. Complex DNA repair mechanisms have

evolved to combat DNA damage from physical or chemical insult, as well as damage from DNA replication. DNA double strand breaks are particularly problematic, as they can result in genomic rearrangements or chromosomal aberrations. The tumor suppressor p53 binding protein (53BP1) is a 1972 residue long protein with a critical role in repairing DNA double strand breaks (DSBs). 53BP1 forms large, phase-separated foci at sites of DSB in response to DNA damage. These foci protect DNA ends from resection, inhibiting homology-directed repair, and recruit downstream effectors that promote nonhomologous end joining. 53BP1 contains an oligomerization domain (OD) and LC8 binding domain (LBD) are important for robust recruitment of 53BP1 to DSBs. LC8 is a small, dimeric hub protein which is known to dimerize over 100 intrinsically disordered client proteins. LC8 binds to 53BP1 multivalently at three sites (QT1, QT2, and QT3) in 53BP1 which all located within residues 1150-1200. The OD is proximal to the LBD, expected to compose residues 1230-1270. While dimeric ODs near LBDs are a common feature of LC8 binding clients, the OD of 53BP1 is a stable trimer. This novel discovery highlights the diverse functions and binding mechanisms that LC8 can participate in. While the 53BP1 LBD alone binds 53BP1 cooperatively and homogeneously, addition of the OD results in a heterogenous mixtures of complexes. Analytical ultracentrifugation reveals large complexes and SEC-MALS shows that these complexes have mass consistent with trimers and dimers-of-trimers of 53BP1. Isothermal titration calorimetry shows divergent binding mechanisms for different LC8 binding sites in 53BP1, and suggests that QT2 is necessary and sufficient for stable dimer-of-trimers formation. QT2 is the only LC8 binding site in 53BP1 containing an S/TQ motif, suggesting it may be phosphorylated by ATM kinase in response to DNA damage. A phosphomimetic of QT2 shows evidence of unstable dimer-of-trimers formation. We propose that phosphorylation of 53BP1 QT2 is a regulatory mechanism, leading to a shift in

53BP1 oligomerization that could lead to improved accumulation of 53BP1 at DSBs.

#26 - MAPPING THE PRECISION SIGNALING MECHANISMS OF ELECTROPHILIC METABOLITES AND DRUGS

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Dr. Jesse R. Poganik, Swiss Federal Institute of Technology Lausanne (EPFL)

Dr. Saba Parvez, Cornell University

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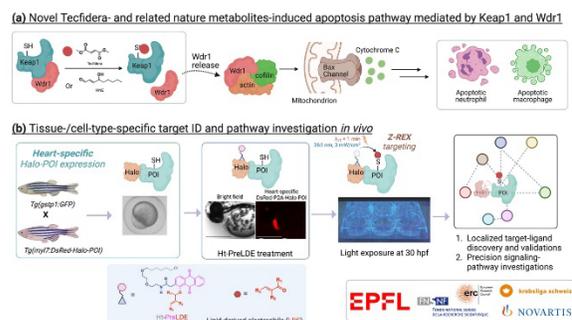
Dr. Marcus J. C. Long, University of Lausanne (UNIL)

Prof. Yimon Aye, Swiss Federal Institute of Technology Lausanne (EPFL)

Despite the importance of reactive electrophilic drugs, identifying their principal targets and pathway mediators remains challenging due to their intrinsic reactivity and lack of genetic tractability in probing such non-enzymatic reactive chemical signaling events. Over the recent years, our laboratory has established unique chemical genetic toolsets to resolve this decades-long challenge in the field through an *on-demand* precision localized reactive-electrophile delivery platform, termed REX technologies. This tool, most recently adapted for use in larval zebrafish (Z-REX), enables the delivery of electrophilic metabolites to specific proteins of interest (POI) in live fish embryos. By controlling the dosage, chemotype, locale, and time of electrophile delivery, Z-REX mitigates off-target effects, metabolic vulnerability, and systemic toxicity commonly encountered following uncontrolled whole-animal electrophile administration.

My ongoing Ph.D. thesis work highlights: (i) Z-REX workflow (Huang, Poganik, *et al. Nat Protoc* 2023), (ii) the latest discoveries uniquely enabled by Z-REX with the focus placed on (A) immune-cell-specific apoptotic mechanisms driven by electrophilic drug Tecfidera: where we discovered a novel Keap1/Wdr1/cofilin-mediated electrophile-signaling axis (Poganik,

Huang, *et al. Nat Commun* 2021); (B) *in vivo* precision mapping of cardiomyocyte-specific electrophile-sensor proteins, and their electrophile-dependent functions in the developing heart (Huang, Chen, *et al.*, 2023, in preparation); (C) electrophile labeling of one zebrafish-Keap1-paralog (zKeap1b) stimulates Nrf2-driven antioxidant response (AR) signaling, while zKeap1a is a dominant-negative regulator of electrophile-promoted Nrf2-signaling. (Van Hall-Beauvais, Poganik, Huang, *et al. Elife* 2022).



#86 - PROBING OF SARS-CoV-2 VIRAL RNA TERTIARY STRUCTURE THROUGH IN VIVO CHEMICAL CROSSLINKING

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Dillon Willis, University of Oregon

Prof. Victoria DeRose, University of Oregon

To improve understanding of the structure and function of the SARS-CoV-2 virus, and to inform the development of therapeutics against COVID19, accurate 3D models of SARS-CoV-2 RNA structures are essential. SARS-CoV-2 RNAs are predicted to form extensive secondary structures, suggesting regions of complex three-dimensional structure, mediated by short- and long-range tertiary interactions that regulate viral replication and transcription. However, computational predictions of tertiary structure have been severely limited by lack of experimental constraints on global RNA-RNA contacts. Our work seeks to enable more accurate 3D modeling of SARS-CoV-2 RNA structures through the identification of tertiary contact points by means of chemical crosslinking.

This technique provides valuable insights into the 3D architecture of RNA, revealing important structural motifs, secondary structures, and spatial arrangements. The DeRose lab has previously shown that cis-diamminedichloroPt(II) (cisplatin) is effective as a crosslinking agent for RNA. Cisplatin and other similar platinum(II)-based reagents can act as robust, selectively reversible, cell-soluble crosslinkers, and can be functionalized with clickable handles for the efficient isolation of crosslinked samples. In particular, the preferential guanine-guanine crosslinking modality of Pt(II) crosslinkers enables the capture of unique RNA-RNA interactions. Recent work has focused on characterizing long-distance interactions involving the untranslated regions of the SARS-CoV-2 genome, which have been found to serve essential functions in transcription. An in vitro analysis of several candidate interactions suggests that several functional RNA-RNA interactions involving the leader transcription regulatory sequence (TRS-L) are energetically accessible at biological conditions in absence of viral or host RNA-binding proteins. Current work makes use of a lab-tractable SARS-CoV-2 minigene containing the UTR sequences to discover novel interactions in an unbiased manner in vivo. This approach makes use of click-capable Pt(II) crosslinking agents, enabling capture and pulldown of viral RNA from its folded state in cells. Identified crosslinks will refine current models of SARS-CoV-2 RNA structures and aid the development of small-molecule therapeutics against the virus. Additionally, the tools and methods developed will be applicable for the structural characterization of emerging SARS-CoV-2 variants and future novel RNA viruses.

#6 - CHEMICAL BIOLOGY AT THE ENVIRONMENTAL MOLECULAR SCIENCE LABORATORY (EMSL), A DOE USER FACILITY

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 Ms. Irlanda Madrano, EMSL, PNNL
 Mr. Phong Huynh, PNNL

EMSL, a DOE Office of Science user facility, offers capabilities to support researchers working in the science areas of DOE's Biological and Environmental Research (BER) program through a competitive grant mechanism, providing state-of-the-art instrumentation and staff expertise at no cost to the researchers. Recently, activity-based probes (ABPs) and associated methods became available as a resource through EMSL to the BER user community. This rapidly expanding capability of EMSL supports the development of new activity-based probes and associated methods focused on plant and microbial systems relevant to the sustainable production of biofuels, chemicals, and biomaterials, environmental microbiology, biosystem design, and more. EMSL's current resources enable activity-based protein profiling, ABP-enabled functional cell sorting and fluorescent imaging, and functional screening libraries. By providing these resources, EMSL aims to increase the use and impact of chemical biology approaches in advancing the BER relevant science. Additionally, EMSL also invests internally to develop novel chemical probes and methods that are offered to EMSL users. In this presentation, the audience will learn ways to partner with EMSL for the development of new chemical probes and related approaches. The presentation will also highlight the recently developed non-canonical amide hydrolase screening library for functional annotation of proteins of unknown function, and the development of photoaffinity probes that mimic terpenes and sugars, aiding in understanding

plant-microbial interactions and biochemical pathways relevant to bioenergy biosystems.

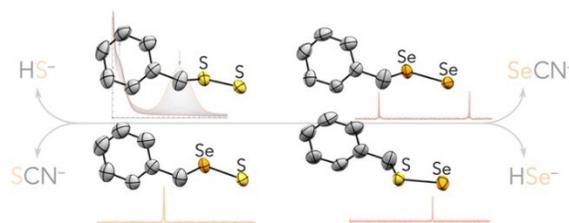
#24 - UNDERSTANDING POST-TRANSLATIONAL MODIFICATION BY REACTIVE SULFUR AND SELENIUM SPECIES THROUGH SYNTHETIC MODELING

Mr. Keyan Li, University of Oregon

Dr. Lev. N. Zakharov, University of Oregon

Professor Michael D. Pluth, University of Oregon

Post-translational modification of cysteines and selenocysteines play key roles in controlling oxidative stress, protein structure, and cellular signaling pathways. Specifically, oxidative modification of thiols (RSH) and selenols (RSeH) by reactive sulfur and selenium species generates reactive alkyl dichalcogenides such as persulfides (RSS⁻), thioselenides (RSSe⁻), and selenosulfides (RSeS⁻), all of which are ubiquitous storage sources for biological S²⁻ and Se²⁻ crucial for various essential physiological roles. Given the highly reactive nature of anionic alkyl dichalcogenides, probing the structure and reactivity of these biologically active species *in vitro* remains challenging. This work describes our efforts in modeling reactive alkyl dichalcogenides using small molecular scaffolds. We show that stabilization of these anionic species using contact-ion pairing interactions enabled the first isolation of RSS⁻, RSSe⁻, RSeS⁻, and RSeSe⁻ with simple steric profiles and discrete in-solution behaviors. Isolated alkyl dichalcogenides were structurally and spectroscopically characterized, and hypothesized biologically relevant reactivities were tested. The first isolation of a RSeSe⁻ provided further implications for perselenidation under both high and low Se concentrations. Overall, our work illustrates new strategies to understand post-translational modification of RSH and RSeH and opportunities in biomimetic modeling and H₂S/H₂Se delivery.



#14 - THE ADRENAL STRESS RESPONSE IS AN ESSENTIAL HOST RESPONSE AGAINST THERAPY-INDUCED LETHAL IMMUNE ACTIVATION

Prof. Xiangnan Li, University of Kentucky

Dr. Ling Guo, University of Kentucky

CAR-T and allogeneic hematopoietic cell transplantation represent major advances in cancer therapy, and have saved the lives of thousands. However, these life-saving therapies are often challenged by lethal immune activation caused by cytokine release syndrome (CRS). Currently, IL-6 antagonists and glucocorticoids (GC) are used for CRS treatment, but some patients respond poorly to treatment. A limitation is that CRS may have already caused irreversible organ injury before the treatment is initiated. Thus, identifying patients at high risk of CRS and providing preventive therapy are in urgent need. We report here that the adrenal stress response, defined by a 5-10-fold increase in induced GC (iGC) production, is an essential host response against lethal T cell activation. We identified scavenger receptor BI (SR-BI), an HDL receptor, as a key regulator for iGC production. Using SR-BI null mice as an adrenal stress response deficiency model, we demonstrate that the adrenal stress response protects against anti-CD3 induced-death through controlling CRS. Conversely, relative adrenal insufficiency (RAI) – the absence of adrenal stress response -- is a risk factor for CRS. Our findings provide a proof-of-concept that diagnosing RAI may help identify patients at risk of CRS, and selective GC therapy for patients with RAI prior to the onset of CRS may reduce mortality from lethal immune activation.

#44 - ROLE OF AMYLOID PRECURSOR PROTEIN-LIKE IN THE CELLULAR IMMUNE RESPONSE IN DROSOPHILA MELANOGASTER

Hannah McClain, Oregon State University

Ashley L. Waring-Sparks, Illinois State University

Dr. Nathan T. Mortimer, Oregon State University and Illinois State University

Amyloid precursor protein (APP) dysfunction is associated with the pathogenesis of Alzheimer's disease in humans. APP is a transmembrane protein which is cleaved by either an α - or β -secretase, followed by γ -secretase. This results in three products: an amyloid intracellular domain (AICD), an extracellular domain, and a small peptide. The intracellular products of both pathways are known transcriptional regulators thought to be involved in immune response. The APP ortholog in *Drosophila melanogaster*, amyloid precursor protein-like (APPL), is cleaved via a conserved pathway. The cleavage products have analogous functions, however the genes effected by AICD transcriptional effectors are unknown. The loss-of-function mutant, *Appl[d]*, features a deletion of the interstitial region of the gene. Previous work has shown that flies with the mutant allele fail to launch an immune response against parasitoid wasps. In nature, *Drosophila* are victims of parasitic infection by several wasp species of the hymenopteran family. Following infection, flies launch an immune response to encapsulate and kill the wasp egg. *Leptopilina clavipes* is an avirulent wasp species that infects *Drosophila*, thus providing a system to study changes in gene expression during a successful cellular immune response. RNA was isolated from naive and *L. clavipes* infected *Drosophila* of both wildtype and *Appl[d]* genotypes for RNA sequencing. The goal of this project was to determine which genes, if any, undergo statistically significant changes in expression between genotypes and infection states. Along with pathway analysis of differentially expressed genes, this project further identifies immune mechanisms influenced by APPL cleavage products. To

examine which genes are affected by the β -AICD transcription factor, analysis of RNASeq data was completed using the Galaxy bioinformatics platform. Differential gene analysis using limma-voom has identified several genes which undergo significant changes in expression.

#70 - PHOSPHATASE AND DENITRASE FUNCTIONS OF PROTEIN TYROSINE PHOSPHATASE RECEPTOR T'S D1/D2 TANDEM DOMAINS

Ms. Sarah McGee, Oregon State University

Mr. Stanislaw Stanisheuski, Oregon State University

Dr. John Wang, Case Western Reserve University

Ms. Yiqing Zhao, Case Western Reserve University

Dr. Richard Cooley, Oregon State University

Dr. Ryan Mehl, Oregon State University

Post-translational modifications (PTMs) are prevalent throughout the human proteome. One of these PTMs, tyrosine nitration induced by oxidative stress, is implicated in various diseases, including ALS and cancer. While this nitration has traditionally been considered irreversible, our recent research reveals that the D2 cytosolic domain of Protein Tyrosine Phosphatase Receptor T (PTPRT) reverses tyrosine nitration. Our study provides evidence of nitrotyrosine removal by the D2 domain and ERK dephosphorylation by the D1 cytosolic domain of PTPRT, highlighting the protein's dual denitrase, phosphatase functionality. Additionally, we provide the first structural insights into the tandem D1 and D2 domains of PTPRT.

#84 - A PLATFORM TO EXPLOIT SPATIAL PRIVILEGE FOR COVALENT INHIBITOR DEVELOPMENT

Mr. José L Montaña, University of California, San Francisco

Dr. Yixin Liu, University of California, San Francisco

Dr. Ed Linossi, University of California, San Francisco

Prof. Natalia Jura, University of California, San Francisco

Prof. Balyn W. Zaro, University of California, San Francisco

The development of covalent kinase inhibitors (CKIs) with high selectivity for their intended target remains a challenge. We previously demonstrated that cysteine reactive CKI selectivity can be significantly improved through electrophilic structure optimization. Specifically, our work took advantage of a spacious cavity adjacent to the reactive cysteine on the target protein of interest. By increasing the steric bulk of the warhead, we maintained efficient engagement and inhibition of our target protein while reducing off-target inhibition through steric exclusion. We hypothesize that there exists a subset of proteins in the human proteome, in particular kinases, that harbor binding sites adjacent to reactive cysteines that are larger or more solvent-exposed compared to others. These “spatially privileged” targets are therefore capable of engaging bulkier CKIs with bulkier warheads, and we propose that this permissiveness can be exploited for improved selectivity in small molecule inhibitors. By integrating chemical biology techniques and taking advantage of recent advances in probe development and chemical proteomics, our work will identify kinases with spatial privilege that could be targeted using CKIs with steric bulk. This work will establish yet another approach in the CKI development pipeline that can be used in conjunction with other existing methods.

#68 - MYPT1 O-GlcNAc MODIFICATION CONTROLS THE SENSITIVITY OF FIBROBLAST SIGNALING FOR CONTRACTION

Ms. Murielle Morales, University of Southern California

Dr. Nichole Pedowitz, University of Southern California

Prof. Matthew Pratt, University of Southern California

Thousands of proteins have been found to be modified by O-GlcNAc, a common glycosylation modification of serine and threonine residues throughout the cytosol and nucleus. O-GlcNAc is

enzymatically added and removed from proteins, making it a potential dynamic regulator of cell signaling. In addition, O-GlcNAc levels are closely tied to nutrient availability, and hyperglycemia in diabetes results in elevated O-GlcNAc. However, compared to other post-translational modifications, relatively few O-GlcNAc regulated pathways have been discovered and biochemically characterized. Using a combination of small molecule inhibitors, 2D and 3D cell cultures, and biochemistry, we have discovered that O-GlcNAc controls the activity of the phosphatase MYPT1. We found that O-GlcNAc on MYPT1 controls the contraction of mouse and primary human dermal fibroblasts initiated by the signaling lipids sphingosine-1-phosphate and lysophosphatidic acid. Specifically, O-GlcNAc on MYPT1 blocks its inhibitory phosphorylation by ROCK and subsequently keeps myosin-II inactive and prevents actin rearrangement. These findings have important implications in our understanding of the role of increased O-GlcNAc in diabetes signaling pathways and contributing to complications in wound healing.

#96 - DETERMINATION OF ACCUMULATION OF MOLECULES IN ESCHERICHIA COLI

George Ongwae, University of Virginia

Liu, Z.¹, Feng, S.², Chordia, M.D.¹, Dalesandro, B.E.¹, Guo, T.³, Dong, J.³, Im, W.², and Pires, M.M.^{1*}

¹University of Virginia

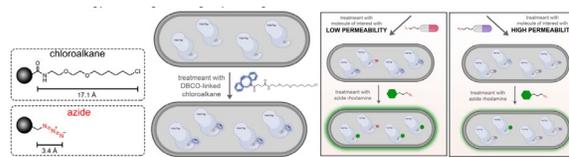
²Lehigh University

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Developing successful agents to combat Gram-negative bacterial pathogens remains challenging due to the inherent resistance caused by their outer membranes that hinder the penetration of antibiotics. A significant bottleneck to drug discovery and development is that few methods exist for measuring the permeation of molecules past the outer membrane. Traditional methods, such as Minimum Inhibitory Concentration (MIC), have limitations in estimating drug accumulation independently from drug potency. Recent insights into small molecule accumulation in

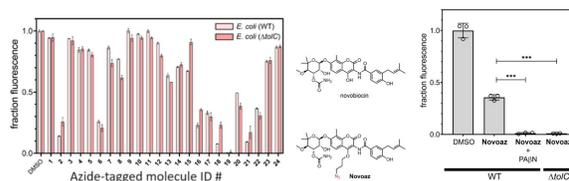
Escherichia coli (*E. coli*) have emphasized the role of primary amines, but these studies are constrained by mass spectrometry methodologies.^{1,2} Although mass spectrometry methodologies offer certain advantages, they also possess inherent limitations, including restricted throughput capacity and an inability to definitively ascertain cytosolic accumulation. Recently, the Kritzer Lab introduced a clever approach for quantifying molecule accumulation in mammalian cells known as the ChloroAlkane Penetration Assay (CAPA); it involves the application of chloroalkane-tagged test molecules (pulse step) to cytosolic HaloTag-expressing mammalian cells.³ Subsequent detection of chloroalkane-fluorophore signals (chase step) reveals the penetration levels. Inspired by the success of CAPA in quantifying molecule accumulation within mammalian systems, we recently adapted this approach to measure the penetration of small molecules within Gram-negative pathogens, an effort we refer to as Bacterial CAPA (BaCAPA).⁴

Despite the wide adoption of CAPA in the measurement of molecule accumulation in mammalian systems^{5,6}, we recognized the potential confounding influence of the 15-atom long chloroalkane tag on penetration analysis in bacteria. In contrast, azides are known for their minimal size and relatively low disruptive impact as biorthogonal tags.⁷ We have, therefore, introduced a robust assay, the Bacterial Azide Penetration Assay (BAPA), for quantitative assessment of small molecule accumulation within Gram-negative bacteria. BAPA employs biorthogonal epitopes anchored within HaloTag-expressing bacteria and measures permeation using azide-bearing test molecules through strain-promoted azide-alkyne cycloaddition (SPAAC). To enable our assay to operate seamlessly with azide-tagged test molecules, we devised a strategy involving HaloTag-expressing bacterial cells.



Specifically, HaloTags undergo an *in situ* reaction with a chloroalkane linked to a dibenzocyclooctyne (DBCO) moiety. These HaloTag-bound cyclooctynes can then be leveraged to gauge the accessibility of azide-bearing test azide-tagged molecules using an *in cyto* strain-promoted azide-alkyne cycloaddition (SPAAC) strategy.⁸ The readout is by an azide-tagged fluorophore in the chase step.

This adaptation allows us to achieve precise measurements of small molecule accumulation compatible with bacterial systems. We demonstrated that BAPA can be leveraged with gene deletion methods to delineate substrates of a major efflux pump of *E. coli*, TolC. This assay offers a facile, high-throughput, and accessible approach, facilitating the permeation analysis of over 1000 molecules.



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#58 - UNDERSTANDING THE UNIQUE NUCLEOLAR STRESS PATHWAY INDUCED BY SMALL-MOLECULE Pt(II) COMPOUNDS AND ITS DEVIATION FROM THE DNA DAMAGE RESPONSE

Ms. Hannah Pigg, University of Oregon
 Ms. Katelyn Alley, University of Oregon
 Mr. Christopher Griffin, University of Oregon
 Mr. Matthew Yglesias, University of Oregon
 Mr. Caleb Moon, University of Oregon
 Ms. Sarah Kraske, University of Oregon
 Prof. Victoria DeRose, University of Oregon

The cellular mechanism by which Pt(II) chemotherapeutics act in cells has been under investigation since the initial FDA approval of cisplatin in 1978. The most widely accepted mechanism by which these compounds work is through DNA crosslinking and subsequent induction of the DNA damage response (DDR) leading to apoptotic cell death. Recent studies indicate that while cisplatin causes cell death through DDR, the related compound oxaliplatin may induce cell death through inhibition of ribosome biogenesis, often referred to as nucleolar stress. The DeRose lab is interested in understanding the mechanisms by which oxaliplatin and related Pt(II) compound derivatives induce nucleolar stress. Structure-function analyses with focus on the diaminocyclohexane (DACH) carrier ligand of oxaliplatin have discovered a limited number of derivatives that cause nucleolar stress. These compounds vary in the initial induction time of nucleolar stress, as well as the overall degree of stress induced. To further confirm that these compounds are working through a unique nucleolar stress pathway, and not DDR, the activation of key DDR proteins including H2AX, ATM and ATR have been studied. It has been found that nucleolar stress inducing-Pt(II) compounds do not cause significant phosphorylation of DDR proteins compared to cisplatin and other known DDR inducing compounds. Additionally, stress inducing-Pt(II)

compounds were still able to induce NPM1 redistribution while the phosphorylation of DDR proteins was inhibited. These studies were carried out across three different cancer cell lines, A549, U 2-OS, and HCT116 cells to confirm that the results were not cell line dependent and did not depend on the cytotoxicity of oxaliplatin compared to cisplatin. Combined, this body of research suggests that DDR may not be a key activator of cell death induced by nucleolar stress inducing-Pt(II) compounds and nucleolar stress is not working downstream of DDR for these compounds. Future studies will focus on further understanding Pt(II)-induced nucleolar stress with the overarching goal of creating more tunable and effective Pt(II) chemotherapeutic drugs.

#82 - CREATING CONJUGATE READY ANTIBODY FRAGMENTS IN E. COLI

Abigail Hope Pung, Oregon State University
 Alex Eddins, Oregon State University
 Dr. Ryan Mehl, Oregon State University

Antibodies and antibody fragments play a crucial role in advancing science and medicine by serving as essential components in protein therapeutics, diagnostics, and research tools. A strategy employed to enhance antibody functionality involves the site-specific encoding of noncanonical amino acids (ncAAs) through Genetic Code Expansion (GCE). This approach eliminates the need to conjugate fluorescent dyes, labels, and drugs to cysteines, thereby expanding the range of incorporation sites. Here we integrated the rapid and bioorthogonal tetrazine ncAAs into E. coli protein expression methods to generate antibody fragments suitable for drug or probe conjugation. We explored the use of solubility tag fusion proteins, various expression conditions, and a recently published E. coli chaperone and disulfide isomerase system. Subsequently, we integrated an improved tetrazine ncAA encoding system, resulting in the synthesis of tetrazine Fabs and

Fab conjugates. This research significantly enhances the methodology and accessibility of producing ncAA-containing antibody fragments, facilitating rapid and complete conjugation of probes to specific antibody sites.

#46 - THE CONFORMATIONAL FLEXIBILITY IN THE IDR OF LATS1 DETERMINES MOLECULAR RECOGNITION

Sanjay Ramprasad, Oregon State University

Lydia Joy Pung, Oregon State University

Evan Hunt, Oregon State University

Prof. Afua Nyarko PhD, Oregon State University

Intrinsically disordered proteins (IDPs), and proteins containing regions of intrinsic disorder (IDRs) are essential components of signaling and regulation networks as their conformational flexibility imbues a single polypeptide with the versatility to facilitate interactions with an extensive variety of ligands and substrates. The dynamic behavior of IDPs (and IDRs) arises from their unique sequence composition (low hydrophobicity, low sequence complexity) that in turn generates an ensemble of interconverting conformers across a relatively flat free energy landscape. A true understanding of an IDPs function therefore requires characterization of their conformational ensembles, at atomic-level resolution, and the timescales they occur, in both the free and bound states of the IDP. Here, using nuclear magnetic resonance (NMR) spectroscopy and isothermal titration calorimetry (ITC), we characterize an approximately 250 residue, intrinsically disordered region (357-604) of the Human Large Tumor Suppressor Kinase I (LATS1). LATS1 is a critical regulatory check point in the conserved Hippo pathway, a global regulator of cell proliferation. Using relaxation (T1, T2) and heteronuclear NOE experiments, we demonstrate that this region of LATS1 is highly dynamic with the notable exception of two rigid "hotspots." The regions of rigidity correspond to two short, linear, Pro-rich motifs (PY1, PY2), found within this stretch, that facilitate interactions with proteins containing single or

tandem WW domains. Unsurprisingly, this region of LATS1 displays unique dynamics upon binding that depends on the identity of the WW domain containing binding partner. When this region LATS1 binds the WW domains of Yes-associated protein 1 (YAP1), its direct downstream effector, we observe reduced conformational flexibility in regions surrounding both PY motifs, as well as in the linker region between suggesting the binding interface spans both PY motifs and a relatively stable complex is formed with single, heavily populated low energy state. In contrast, when this region of LATS1 binds the WW domains of the upstream regulator KIBRA, we observe enhanced conformational flexibility surrounding PY2 and the linker region in between the motifs, while the dynamical behavior distal to PY2 remains relatively unperturbed, suggesting the binding interface is primarily located proximal to PY2 and that the complex is potentially in exchange between multiple populated states of similar free energy. Within signal transduction networks, information flow involves a cascade of binding or covalent modification processes, with each step in the cascade directly affecting the subsequent step. Ultimately, molecular recognition is determined, and information is transduced by the changes in the distribution of protein ensembles that arise from these processes.

#72 - HARNESSING RNA-PROTEIN INTERACTIONS TO DEVELOP A NOVEL BACTERIAL BIOSENSING SYSTEM

Mr. Tyler J. Simons, University of Utah

Dr. Ming C. Hammond, University of Utah

Riboswitches have previously been fused to dye-binding RNA aptamers to produce RNA-based fluorogenic (RBF) biosensors. Upon the riboswitch aptamer binding its respective signaling molecule, the RNA changes conformations to bind the fluorescent dye, producing a bright fluorescent signal detectable both *in vivo* and *in vitro*. These genetically encodable biosensors have been revolutionary,

but these tools also have drawbacks. For example, the dye must be added exogenously, and the fluorescent dye may exhibit high background fluorescence in certain circumstances.

A fluorogenic protein system was previously developed to visualize mRNA localization in mammalian cells,¹ which uses an RNA-binding protein (tat) to allow TAR RNA to conditionally conceal a degron tag appended to a fluorescent protein to essentially toggle an FP signal. Unlike RBF biosensors, this system is fully genetically encodable and displays very low background fluorescence. However, the published fluorogenic protein system uses a C-terminal degron tag only recognized by a mammalian proteasomal degradation pathway and is therefore not effective in prokaryotes. The goal of this project is to develop and optimize a similar conditional degron system for bacterial model organisms using the tat-TAR pair and a bacterial degron tag.

Both N- and C-terminal degron tags have been tested for degradation, with a C-terminal tag displaying TAR RNA rescuing the protein from degradation. When the first C-terminal degron we tested did not have the desired degradation of Venus, we pursued other degron tags. The next C-terminal degron that we pursued had excellent degradation when appended to Venus. The tat peptide was then inserted into the latter degron with designs minimizing the degron tag for more likely rescue by TAR RNA. Three designs were successfully degraded, with the best degrader also demonstrating an 85-110% rescue of Venus by TAR RNA, or a 60-fold increase in fluorescence compared to a control RNA. Adapting this conditional degron system into a TAR-based small molecule or small RNA (sRNA) biosensor² has proven difficult due to the high stability of the TAR RNA hairpin. Truncations and mutations have been made to TAR RNA and progress on the development of TAR-based biosensors will be reported at this

conference to display the versatility of this novel prokaryotic biosensing system.

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#64 - HOW DO THE BIOPHYSICAL PROPERTIES OF AMYLOID β_{42} CONTRIBUTE TO THE PATHOGENESIS OF ALZHEIMER'S DISEASE?

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 Nguyen Le, Illinois State University
 Ashley L. Waring, Illinois State University
 Nate Mortimer, Oregon State University

Since the development of the amyloid hypothesis in 1991, extensive work has been carried out to understand how the peptide amyloid β contributes to the development of Alzheimer's Disease (AD). The early amyloid hypothesis focused on amyloid plaques, commonly characterized as a prominent feature of AD, as the primary driver of AD pathogenesis. More recent evidence indicates that these amyloid plaques are relatively benign and found in brains of people without AD. These observations have led to a new hypothesis, coined the "amyloid cascade hypothesis", positing that soluble amyloid oligomers formed in the intermediate stage of aggregation are likely the cytotoxic component that leads to the development and progression of Alzheimer's Disease. Many questions surround the importance of size and structure of these oligomers and how these factors relate to neurotoxicity. Recent work points to inflammation as a key mediator of the toxicity of A β oligomers, but both the mechanism and the consequences of this inflammation remain unknown. Recent evidence demonstrates that A β may stimulate cytokine pathways to promote a pro-inflammatory response, and additional data

suggest that the A β -mediated activation of glial cells plays an essential role in Alzheimer's Disease pathogenesis.

We hypothesize that the natural function of A β is to serve as an opsonin, and that its aberrant aggregation serves to activate the immune cells that are responsible for the inflammatory response seen in AD patients. We further hypothesize that there are unique biophysical properties of A β oligomers responsible for the hallmark symptoms of AD. Difficulties have arisen in studying these amyloids as they are intrinsically disordered with a marked level of instability, leading to challenges expressing and purifying these proteins in physiologically relevant conditions, significantly limiting the advancements in understanding the mechanisms behind this disease. A β toxicity appears to be linked to the presence of an alpha sheet motif, however, the role that this alpha sheet motif contributes to inflammation is still unknown.

To test our hypotheses, we plan to use transgenic *Drosophila melanogaster* lines that express human A β with mutations relevant to familial mutations observed in AD patients, which have been found to exhibit the hallmarks of AD: namely, the neurodegeneration and memory impairments typical of AD. By utilizing *Drosophila*, we will couple in vivo cognitive assays with the robust biophysical characterization of these oligomers to advance our understanding of the role A β plays in AD pathogenesis. This multi-faceted approach should allow us to develop a deeper understanding of A β oligomers in a physiologically relevant manner and allow us to understand how each of these oligomer species contributes to the development of symptoms experienced in individuals living with Alzheimer's Disease.

#76 - MONITORING THE OUTCOMES OF GENETIC MANIPULATIONS AND HIGHLY HOMOLOGOUS ENGINEERED PROTEINS IN SINGLE MAMMALIAN CELLS

Stanislau Stanisheuski, Oregon State University

Kavi Vaidya, Oregon State University

Arpa Ebrahimi, Oregon State University

Alex Eddins, Oregon State University

Dr. Liping Yang, Oregon State university

Dr. Claudia S. Maier, Oregon State University

Introduction: Recent developments in automated single cell and liquid handling improved precision of sample preparation and enabled single cell proteomics. Most current SCP efforts focus on obtaining comprehensive proteomic profiles of cells studied. However, there are many cases where there is a need to focus on evaluating individual low abundant proteins. Especially challenging is distinguishing minor differences in proteins with a significant overlap in sequence, e.g. isoforms, artificially engineered proteins, or proteins containing PTMs. Due to high sequence similarity, such tasks cannot be solved by competing techniques like mass cytometry and scRNAseq. In this study, we demonstrate tracking the consequences of genetic manipulations and differences in expressed engineered proteins at the single cell level.

Methods: HEK293 cells expressing enhanced Green Fluorescent Protein (eGFP), HEK293T cells producing superfolder Green Fluorescent Protein (sfGFP), and HEK293A cells transiently transfected with sfGFP-bearing plasmid were used in this study. Single cells were dispensed by a microfluidic inkjet dispenser (a HP D100 digital dispenser) into wells of a 384-well plate. Reagents necessary for trypsin digestion – DTT, IAM, and sequencing-grade Trypsin – were also added using the inkjet dispenser. Samples were analyzed using an Orbitrap Fusion Lumos coupled with either an Acquity M-class UPLC or Neo Vanquish UPLC. Single cell optimized LC-MS methods, featuring low eluent flowrate,

advanced decision-making trees, elevated AGC levels and injection times for both MS1 and MS2 events, were used for analysis.

Preliminary Data: Collected data show that it is possible to distinguish between sfGFP, eGFP, and transiently transfected sfGFP on a single cell level. Sequence overlap between the sequences of proteins in this study was >98%. Differential tryptic peptides corresponding to the same part of protein sequence were different in only one or two amino acids. Protein levels varied significantly depending on what method was used for the genetic manipulation. Furthermore, it is worth noting that unlike in bacterial cells, in eukaryotic cells expression levels of proteins of interest are generally quite low, making the single cell analysis even more challenging. A proteomic profile covering around one thousand proteins per cell was collected for every single cell. Although the used cell lines are the descendants of the same parent HEK293 cell line, the single cell proteome coverage was enough to clearly differentiate them using PCA and to highlight certain cell proteome changes caused by the genetic manipulations.

Novel Aspect: Tracking the consequences of genetic manipulations and differences in expressed engineered proteins at the single cell level.

#28 - PHOSPHORYLATION OF THE SR-RICH REGION OF THE SARS-CoV-2 NUCLEOCAPSID REGULATES SELF-ASSOCIATION AND RNA INTERACTIONS

- Ms. Hannah Stuwe, Oregon State University
- Dr. Patrick Reardon, Oregon State University
- Ms. Sahana Shah, Oregon State University
- Dr. Zhen Yu, Oregon State University
- Ms. Kaitlyn Hughes, Oregon State University
- Prof. Elisar Barbar, Oregon State University

The nucleocapsid protein (N) of SARS-CoV-2 is essential for virus replication, genome packaging, and maturation. N is comprised of two folded

domains that are separated by a highly conserved, disordered, Ser/Arg-rich linker, followed by self-associating Leu-rich helix, and flanked by disordered tails. Using NMR spectroscopy and analytical ultracentrifugation we show that concentration dependence self-association is localized to the Leu-rich helix and that phosphorylation at Ser188 in the Ser/Arg-rich region promotes dissociation of the helix. NMR and gel shift assays demonstrate that the linker binds viral RNA, but this binding is dampened by phosphorylation, whereas RNA binding to the full-length protein is not significantly affected due to strong interactions with the other RNA binding domains. In contrast, phase separation with RNA is reduced upon phosphorylation in both the linker and full-length proteins. We attribute the changes in phase separation to weakening of the linker helix self-association upon phosphorylation. These data provide a structural mechanism that explains how phosphorylation in the disordered linker could affect protein-protein interactions, RNA-protein interactions, and liquid-liquid phase separation.

#22 - ACTIVATION MECHANISM AND ROLE OF LIPIDS IN OSCA GATING

- Jed Syrenne, Oregon Health & Science University
- Aidan Berryman, Oregon Health & Science University
- Dr. Steve Reichow, Oregon Health & Science University
- Dr. Swetha Murthy, Oregon Health & Science University

OSCA/TMEM63s are a family of mechanically activated (MA) ion channels, implicated in human hypomyelinating disorders, pain, and cognitive dysfunction. To understand these channels' pathophysiology, we must appreciate the underpinnings of their molecular mechanisms. We have yet to elucidate their mechanisms of inactivation, gating, or the factors of their lipid environment which affect tension sensing. Recent studies have yielded closed-state structures of OSCA1.2. These structures

revealed a putative sterol binding site buttressing the channel, opening up the possibility of functional modulation by the lipid environment. I propose that OSCA mechano-activation is modulated by sterols via allosteric mechanisms or by altering the biophysical properties of the membrane environment. I will utilize patch clamp electrophysiology, structural studies, and photoactivatable-lipid tools, to understand the effect of lipids on OSCA/TMEM63 gating and derive their activation mechanism. My studies will unravel how tension activates these channels, facilitating a deeper understanding of their in vivo mechanosensation roles.

#56 - BIOENGINEERING OF SITE-SPECIFIC ANTIBODY LABELING FROM BASIC RESEARCH TO SPATIAL OMICS PLATFORMS

Dr. Kai Tao, Oregon Health & Science University

Dr. Matthew J Rames, Oregon Health & Science University

Dr. MingChong Dai, Oregon Health & Science University

Dr. Gulsu Sener, Oregon Health & Science University

Mr. Samuel Olson, Oregon Health & Science University

Dr. Srivathsan Ranganathan, Oregon Health & Science University

Dr. Sila Ozdemir, Oregon Health & Science University

Mr. Adam Oken, Oregon Health & Science University

Dr. Michael Brasino, Oregon Health & Science University

Mr. Randy Armstrong, Oregon Health & Science University

Dr. Yu-Jui Chiu, Oregon Health & Science University

Prof. Ece Eksi, Oregon Health & Science University

Prof. Bruce Branchaud, Oregon Health & Science University

Prof. Sadik Esener, Oregon Health & Science University

Over the last few years, spatial multi-omics platforms have emerged as a powerful tool for characterizing cancer biology in multiple dimensions. Notably, these systems typically rely

on multiplex immunolabeling. Conventionally, multiplexing is accomplished through carefully selecting primary antibodies from different animal species, and in combination with their corresponding polyclonal secondary antibodies. However, given the practical complication related to the species availability of primary antibodies, the multiplexing labeling is still under constraint due to the necessity for matched secondary species labels and the availability. For decades, non-specific labeling of primary antibodies through amine- or thiol-reactive chemistries was widely performed. Evolving research and commercial products have sought to overcome this antibody-based multiplexing limitations by developing enzymatic reaction-directed chemical reaction or antibody targeting proteins that could contain affinity to the Fc or Fab regions of antibodies. However, these methods mentioned above have substantive limitations and side-effects on affinity, conjugation number, buffer incompatibilities, storage incompatibilities, insufficient conjugation, and uncontrolled stoichiometric labeling. Herein, we introduce an efficient, sustainable, reproducible site-specific antibody covalent labeling strategy through engineering anti-IgG secondary nanobodies. These infer the stability, high-affinity of species specific and easy-to-produce IgG binders with the permanence of controlled activation for proximity-driven intermolecular covalent conjugation, with a promise for efficient multiplexity. This labeling technology addresses antibody labeling challenges through currently available approaches, and would substantially enhance multiplexity in a wide variety of research and clinical settings.

#100 - PLAYING "REDOX HOT POTATO": DISULFIDE TRANSFER BETWEEN Γ -CRYSTALLINS IN THE AGING, PRE-CATARACTOUS LENS

Dr. David Thorn, Harvard University

Dr. Eugene Serebryany, Harvard University

Prof. Larry David, Oregon Health & Science University

Prof. Kirsten Lampi, Oregon Health & Science University

Prof. Eugene Shakhnovich, Harvard University

Oxidation of susceptible eye lens crystallin proteins is a key process in lens opacification and the development of cataract, which increases with age as glutathione levels in lens fiber cells are depleted, especially in the nuclear region. Here, the cysteine-rich and abundant γ -crystallins may provide the last line of defense against oxidative protein misfolding and aggregation by serving as redox sinks. Previous work from our laboratory [1] demonstrated in vitro the transfer of a disulfide bond from one γ -crystallin protein to another, i.e., from wildtype γ D-crystallin (γ D) to its destabilized tryptophan-oxidation mimicking variant, W42Q, a redox reaction that initiates an oxidative aggregation cascade. In the present study, we explored whether this disulfide oxidoreductase-like activity may be exhibited by other γ -crystallin isoforms, in particular, γ S, which contains a CXCXC motif (residues 22-26) and readily forms disulfide-linked dimers via the highly solvent-accessible C24. Indeed, we found untreated γ S WT triggered the aggregation of γ D W42Q to a degree comparable to oxidized glutathione (Fig. 1). Disulfide-linked dimers of γ S had a less pronounced effect on γ D W42Q aggregation. Moreover, both fully reduced γ S WT and a C24S mutant were completely inert. The prerequisite of C24 for disulfide transfer suggests that it occurs between one or more conformationally strained, intramolecular disulfides in the CXCXC motif of γ S. Our findings add to a growing body of work suggesting that γ -crystallins participate in disulfide exchange in the aging, glutathione-depleted lens to modulate oxidative protein aggregation and the progression of age-related nuclear cataract.

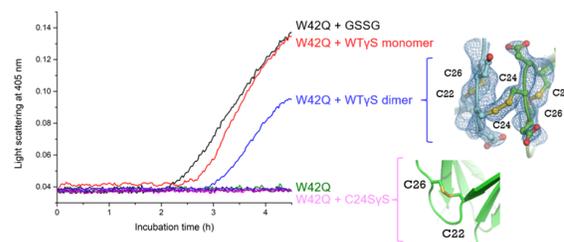


Figure 1. Turbidity assay showing that oxidized γ S WT, but not γ S C24S, can induce the oxidative aggregation of γ D W42Q, likely due to the lower redox potential (greater conformational stability) of the C22-C26 disulfide bond in γ S C24S.

[1] Serebryany E, Yu S, Trauger SA, Budnik B, Shakhnovich EI. Dynamic disulfide exchange in a crystallin protein in the human eye lens promotes cataract-associated aggregation. *J. Biol. Chem.* (2018) 293, 17997-8009.

#36 - SERINE RECOMBINASE-ASSISTED GENOME ENGINEERING (SAGE) ALLOWS HIGH-THROUGHPUT GENETIC CODE EXPANSION IMPLEMENTATION AND OPTIMIZATION IN NON-MODEL MICROORGANISMS.

Dr. Elise Van Fossen, Pacific Northwest National Lab

Mr. Andrew Frank, Pacific Northwest National Lab

Mr. Andrew Wilson, Pacific Northwest National Lab

Mr. Carter Bracken, Pacific Northwest National Lab

Dr. Stephanie Thibert, Pacific Northwest National Lab

Dr. Joshua Elmore, Pacific Northwest National Lab

Genetic code expansion (GCE) gives organisms the ability to incorporate chemically diverse 'non-canonical' amino acids (ncAAs) into proteins, imbuing them with new-to-nature functionalities. The implementation of GCE in microbial engineering space grants us sophisticated chemical biology methods to interrogate the complex biological systems that govern microbial functions relevant to human health (biofilm formation, pathogenicity) along with a new repertoire of chemical functionality for microbe-produced natural products and biocontainment. However, to successfully maintain GCE in a living organism, the orthogonal translational components must be carefully tuned to maximize GCE output while limiting toxicity and the synthetic biology footprint to the hosting organism. This combinatorial engineering challenge has largely limited bacterial GCE implementation to

Escherichia coli for recombinant protein production, severely hampering its use in more diverse microbial environments. To overcome this, we used a multiplexed version of the host agnostic Serine recombinase-assisted Genome Engineering toolkit (mSAGE) to install GCE components in multiple non-model bacterial species (including one environmental isolate) and have seen robust production of GCE-dependent green fluorescent protein in these strains. With the high chromosomal integration efficiency of mSAGE, we have generated combinatorial pooled expression variant libraries for each component of the GCE machinery for the selection of strains with the highest production of GCE-dependent GFP while minimizing toxicity. We next plan to apply our GCE/SAGE system to generate GCE strains of P. aeruginosa to study pathogenicity along with select rhizosphere bacteria to develop GCE-dependent biocontainment strategies.

#74 - THE GCE4ALL RESEARCH CENTER: PROMOTING YOUR ABILITY TO USE GENETIC CODE EXPANSION IN YOUR RESEARCH

Dr. Kari van Zee, Oregon State University
 Prof. P. Andrew Karplus, Oregon State University
 Dr. Kayla Jara, Oregon State University
 Dr. Bettye L.S. Maddux, Oregon State University
 Prof. John D. Lueck, University of Rochester Medical Center
 Prof. Richard B. Cooley, Oregon State University
 Prof. Ryan A. Mehl, Oregon State University
 Genetic Code Expansion (GCE) is a powerful but highly underused technology for the specific placement of chemical groups in proteins. GCE rewires translation, allowing site-specific incorporation of non-canonical amino acids (ncAAs) at genetically encoded sites during protein synthesis. Our mission at the Oregon State University GCE4All Research Center – funded by the Biomedical Technology Optimization and Dissemination program of the National Institute of General Medical Sciences –

is to optimize and disseminate GCE tools so they can be more widely used to reveal mechanisms of health and disease and to develop protein diagnostics and therapeutics.

Our two broad focus areas are: **Bioorthogonal ligations** and **Probes and Post-translational modifications**

The Center “road tests” developing GCE technologies through formal collaborations with selected NIH-funded research groups in order to solve challenging biomedical research problems that cannot be addressed by conventional strategies.

#48 - CHEMICAL PHOTOSWITCHES TO REVERSIBLY MANIPULATE CANNABINOID SIGNALING IN PANCREATIC BETA-CELLS

Alexander E. G. Viray, Oregon Health & Science University

Dr. Roman Sarott, ETH Zürich

Dr. Patrick Pfaff, ETH Zürich

Miroslav Kosar, ETH Zürich

Dr. Erick Carreira, ETH Zürich

Dr. James A. Frank, Oregon Health & Science University

The cannabinoid receptor CB2 is an inhibitory class A GPCR and classic receptor of the endocannabinoid system (ECS). The ECS can be found in tissues that regulate glucose homeostasis, including the pancreatic islet. While CB2 is predominantly in the peripheral immune tissue, its role in mediating b-cell and islet function remains poorly understood. Synthetic cannabinoid ligands, like HU308, can selectively activate CB2 and can be a promising therapeutic target. The promiscuity of ligands, enzymes, and receptors of the ECS, coupled with the hydrophobicity of cannabinoid ligands, poses diffusion limitations which challenge our ability to modulate cellular excitability with spatiotemporal precision. To this end, we developed photoswitchable cannabinoids whose signaling activities can be tuned in a light-dependent manner. Photoswitchable analogs of

HU308 were synthesized to place CB2 signaling under optical control. The probes were evaluated in excitable cell types through live Ca^{2+} imaging which shed light on novel effector pathways by which these GPCRs can affect excitability. In AtT-20 cells overexpressing CB2, our probe stimulated a robust Ca^{2+} transient when photoswitched with UV light through the non-canonical CB2- G_q -coupled-PLC pathway. However, in the pancreatic b-cell line INS-1 832/13, a pharmacological screen demonstrated that the mechanism for the robust Ca^{2+} response when photoswitched was not via CB2, but rather through the involvement of Ca^{2+} release activated Ca^{2+} (CRAC) channels. Current applications of this probe *in vitro* are underway to see if this effect holds true in primary pancreatic islets. Future applications of the probe will include whole-cell patch-clamp electrophysiology in pancreatic b-cells and monitoring changes to glucose-stimulated insulin secretion (GSIS) via insulin assays, which could help interrogate the role of CB2 and CRAC channels in cellular signaling and disease.

#8 - LEVERAGING PLATINUM-PROTEIN INTERACTIONS TO OVERCOME CHEMORESISTANCE

Prof. Fang Wang, University of Rhode Island

A common mechanism by which cancer cells acquire resistance to chemotherapeutics is through the overexpression of efflux pumps. However, platinum anticancer agents that crosslink DNA and interact with proteins are poor efflux pump substrates. We have designed bifunctional drug conjugates by tethering a platinum pharmacophore to doxorubicin, a prototypical efflux pump substrate. These drug conjugates retain the anticancer activity of doxorubicin and exhibit a remarkable ability to both circumvent drug efflux and delay the acquisition of drug resistance. Our mechanistic studies indicate that these drug conjugates overcome resistance through covalent platinum-protein interactions, leading to significantly

improved drug retention and alteration of subcellular drug distribution. This novel application of platinum as an anti-resistance modulator presents a generalizable strategy for augmenting conventional chemotherapeutics.

#10 - CHEMICAL PEPTIDE “CAPS” – AN ENTRY TO UNCOVER NEW MAMMALIAN SIGNALING PEPTIDES

Ms. Amanda L. Wiggenhorn, Stanford University

Ms. Hind Z. Abuzaid, Stanford University

Dr. Laetitia Coassolo, Stanford University

Ms. Veronica L. Li, Stanford University

Ms. Julia Tanzo, Stanford University

Dr. Wei Wei, Stanford University

Dr. Xuchao Lyu, Stanford University

Prof. Katrin J. Svensson, Stanford University

Prof. Jonathan Z. Long, Stanford University

Peptide hormones and neuropeptides are signaling molecules that control diverse aspects of mammalian homeostasis and physiology. In this presentation, I will provide evidence for the endogenous presence of a sequence diverse class of blood-borne peptides that we call “capped peptides.” Capped peptides are fragments of secreted proteins and defined by the presence of two post-translational modifications – N-terminal pyroglutamylation and C-terminal amidation – which function as chemical “caps”. Capped peptides share many regulatory characteristics in common with other signaling peptides, including dynamic physiologic regulation. One capped peptide, CAP-TAC1, is a tachykinin-like molecule and a nanomolar agonist of tachykinin receptors. A second capped peptide, CAP-GDF15, is a previously unknown 12-mer peptide cleaved from the prepropeptide region of full-length GDF15 that, like the canonical GDF15 hormone, also reduces food intake and body weight. In summary, I will present how capped peptides are a potentially large class of signaling molecules with potential to broadly regulate cell-cell communication in mammalian physiology.

#52 - PROBING THE SECONDARY AND TERTIARY STRUCTURE OF THE SARS-CoV-2 5'-UTR

Mr. Dillon Willis, University of Oregon

Mr. Ethan Kimmett, University of Oregon

Prof. Victoria J. DeRose, University of Oregon

SARS-CoV-2, the causative virus behind COVID-19, has a highly conserved 5'-untranslated region (UTR) that could serve as a valuable target for drug design. Our work focuses on creating detailed information about the structure of the 5'-UTR using a variety of chemical crosslinking strategies. One portion of the 5'-UTR, stem loop 5 (SL5), has served as a starting point for this project. SL5 contains the start codon for initial translation of the viral polyprotein, and the RNA subdomain is predicted to be highly structured. We find that when synthesized in vitro, SL5 self-cleaves in the presence of Mg²⁺. Current work is aimed at characterizing this unexpected activity and determining if it is conserved in vivo and in larger portions of the UTR RNA.

#98 - TOWARDS DEVELOPMENT OF A NOVEL 'BIO-TAG' FOR CRYO-EM STUDIES BASED ON THE SMALL, ELECTRON DENSE PROTEIN CSP1

Ms. Weekie Yao, Oregon Health & Science University

Mr. Adam C. Oken, Oregon Health & Science University

Dr. David L. Farrens, Oregon Health & Science University

Small (< 100 kDa) proteins are especially challenging to study by single-particle cryo-EM techniques - their low overall electron density results in a low signal to noise ratio in micrograph images and makes them difficult to identify and analyze. To address this problem, we are testing ways to increase protein contrast in EM images.

Our focus has been on Csp1 (Copper storage protein 1), a small (52 kDa) tetrameric protein that binds up to 52 copper ions. We tested if these bound metals could enhance protein

contrast in raw EM micrographs, potentially making Csp1 an electron-dense tag that could facilitate structural studies of small proteins by single-particle cryo-EM techniques.

Our results show that copper-bound Csp1 exhibits excellent particle contrast in cryo-EM micrographs, even without the use of phase plates. We find copper appears to enhance the resolution of the obtained cryo-EM map for Csp1 (2.98 Å for copper-bound Csp1 vs. 3.55 Å for apo-Csp1).

We also tested if Csp1 could be used as a "tag" or scaffold to help determine the structure of other proteins bound to it, specifically, a Fab antibody fragment, and a small membrane protein. While our results show Csp1 appears to help with particle alignment for these proteins, the flexibility in linkage between Csp1 and bound proteins cause heterogeneity in the dataset which hinders overall structural analysis.

In summary, Csp1 is a promising candidate as an electron-rich 'bio-tag' tag, that with further optimization, can facilitate cryo-EM analysis of small proteins.

#66 - INHIBITION OF RRNA TRANSCRIPTION BY PLATINUM (II) COMPOUNDS

Matthew Yglesias, University of Oregon

Dr. Emily Sutton, University of Oregon

Prof. Victoria De Rose, University of Oregon

Platinum (II) compounds represent a major class of chemotherapeutic agents used in cancer treatment regimens today. However, despite their ubiquitous use the mechanism and targets of these compounds are not fully understood. These compounds are thought to kill rapidly dividing cancer cells by forming cross-links with genomic DNA that interfering with DNA replication and lead to cell death through the DNA Damage Response. This rational led to the design of carboplatin (1989) and oxaliplatin (1996), which were intended to improve on the efficacy and reduce side effects. More recently

we and others have shown that, unlike cisplatin, oxaliplatin induces cell death by disruption of ribosome biogenesis, or the process of making ribosomes. Oxaliplatin has been further found to disrupt early pre-rRNA synthesis and induce a nucleolar stress response, a well-known marker for disruption of ribosome biogenesis; however, the molecular mechanisms for these two processes and their connection are not well understood. rRNA transcription by RNA polymerase I (Pol I) which is highly conserved in eukaryotes and is often deregulated in cancers making it an attractive target for chemotherapeutics. The primary goal of this project is to elucidate the mechanism for inhibition of rRNA synthesis by oxaliplatin by applying a Chromatin immunoprecipitation sequencing (ChIP-seq) to measure the occupancy of the Pol I transcription complex along the rDNA gene to provide a molecular understanding of how oxaliplatin disrupts ribosome biogenesis. Broader implications of this proposed work would provide insight into nucleolar function and benefit the design of new anti-cancer drugs.

#85 - ACCESSIBLE GENETIC CODE EXPANSION WITH TOOL KITS FOR SELECTING YOUR OWN NONCANONICAL AMINO ACYL-TRNA SYNTHETASES

Nathan Alexander, Oregon State University

Dr. Yogesh Gangarde, Oregon State University

Dr. Richard Cooley, Oregon State University

Dr. Ryan Mehl, Oregon State University

Genetic Code Expansion (GCE) has transformative potential in protein engineering, cellular studies, and therapeutic applications. At the heart of GCE there is an orthogonal tRNA-synthetase (RS/tRNA) pair customized for a specific noncanonical amino acid (ncAA) which allows for site specific installation of the ncAA into proteins in many living organisms. These ncAAs have diverse applications including bioorthogonal ligation, fluorescence labeling, photocrosslinking, post-translational modification mimicry, and spectroscopic probing. The creative limits on what ncAAs can be encoded to generate new protein function seems to be limited only by the imagination of the chemist and access to the directed evolution process for tRNA/RS pairs. Here for the first time we provide access the “secret sauce of GCE”, aka the selection kit. Here the GCE4All national center provides the complete genetic libraries and selection plasmids needed for everyone to select tRNA/RS for their own ncAAs. The chemical complexity of desired ncAAs varies widely. Our study focused on Methanomethylophilus alvus (Ma) pyrrolysyl-RS (Pyl-RS), an active site adaptable to a wide range of ncAAs. We applied diverse selection pressures to two large libraries to identify Ma RS variants compatible with tetrazine-3.0 butyl, various fluorinated phenylalanines, and cyclopropenone-lysines (CpOKs). Our hypothesis was that varying selection pressures in a template selection workflow would yield efficient RSs. We utilized a life/death double sieve approach in our selection process, using a TAG site within an antibiotic marker for positive selection and a toxic protein,

barnase, with TAG codons for negative selection. Efficiency was assessed by monitoring super-folder GFP (sfGFP) fluorescence in culture. Lowering ncAA concentration and increasing life/death pressure during repeated tetrazine-3.0 butyl positive selections led to more efficient RSs than those selected under less stringent conditions. A double-sieve selection for pentafluoro phenylalanine, followed by fluorescence-activated cell sorting (FACS) after expression with various fluorinated phenylalanines, generated RSs compatible with ncAA phenylalanines of varying fluorination degrees. A structurally informed Ma RS library designed for elongated lysine-like structures, achieved by shuffling active site residues, produced RSs selective for CpOK-Amide and CpOK Carbamate with moderate efficiency and high specificity. In summary, our study demonstrates that Ma-Pyl RS adaptability to diverse ncAAs can be improved by varying selection pressures to accommodate the demand for diverse ncAAs. Further research is needed to evaluate Ma-RS activity beyond host metabolism and to refine selection processes for challenging or toxic ncAAs. This work enhances our understanding of GCE's potential applications in the study of proteins and cellular processes.

#41 - INVESTIGATING THE ROLE OF SH3BP2 IN CREATING LOCAL HETEROGENEITY OF PROTEIN CONCENTRATION DURING PROTRUSION FORMATION USING OPTOCHEMICAL PROBES.

Ms. Kristina Bayer, Max Planck Institute for Medical Research, Heidelberg

Prof. Shige H. Yoshimura, Kyoto University, Kyoto

Dr. Richard Wombacher, Max Planck Institute for Medical Research, Heidelberg

Contrary to “textbook biology” cellular signalling cascades do not exist in a void, nor are they neatly segregated from other, potentially opposing cascades. In a cellular environment, signalling cascades often share crucial downstream effectors. Actin signalling, in

particular protrusion signalling – lamellipodial and filopodial extensions – is one pertinent example of this signalling promiscuity.

Activation of Cell division control protein 42 homolog (Cdc42) – the member of the Rho family of small guanosine Triphosphatases (GTPases) responsible for filopodia initiation – for example, leads to activation of Rac Family Small GTPase 1 (Rac1), which is canonically thought of as the protein responsible for lamellipodia. Other crucial effectors such as Arp2/3 are known to be necessary for lamellipodia-related actin filament branching, yet have also been shown indispensable for filopodia initiation, as well as being present during focal adhesion signalling. Similarly, Cdc42 interacting protein 4 (CIP4) has been shown to be essential for clathrin-mediated endocytosis, while also being a dynamic scaffold for lamellipodial extensions.

Were the dynamic, membrane-less compartmentalization of promiscuous with members specific to a cascade not spatio-temporally controlled, i.e. were mechanisms creating local heterogeneities in protein concentration not available, actin signalling fate were not able to be controlled by the cell. Not only is this unlikely, given its importance to cellular survival, but dynamic induction of local heterogeneity might actually be of benefit to highly dynamic signalling cascades.

This work has identified a candidate enforcer of local heterogeneity during lamellipodial extension – SH3 binding protein 2 (SH3BP2) and seeks to validate its role by manipulating its local concentration using lipid-like, membrane-localizing opto-chemicals, based on the Trimethoprim-eDHFR - tag. The compound, upon addition to cells, autonomously localizes in the inner leaflet of the PM, where it can recruit ¹⁴C-eDHFR-tagged proteins in a constitutive, or conditional – if photocaged – fashion. By emulating physiological recruitment triggers, interrogation of downstream effects of the

cytosol-to-plasma membrane translocation event, itself, is made possible.

Through a combination of confocal fluorescence microscopy and *in vitro* techniques the localized abrupt increase of SH3BP2 concentration at the plasma membrane (PM) has thus been shown to increase colocalization of active Rac1, as well as Arp2/3, with actin while having no effect on members specific to the filopodial cascade. This, in turn increases the likelihood of lamellipodial effectors interacting with one another, while decreasing their likelihood of interaction with filopodial members, which is hypothesized to support signalling flux through the lamellipodial cascade. *In cellulo*, this presence in proximity of the PM, and SH3BP2's subsequent ability to engage with its' interactome, has been found to be controlled by PIP₃-transients. Moreover, recruitment of SH3BP2 to specific sub-cellular regions of the PM was found to result in a PIP₃-dependent, self-amplificatory mechanism leading to ever more localized SH3BP2 recruitment.

Taken together, data from the lab lends considerable support for the hypothesis that dynamic, localized recruitment via PIP₃-transients of SH3BP2 serves as the nucleator of an increase in lamellipodial effectors at the locus of recruitment, which in turn ensures maintenance of signalling faithfulness, in spite of effector promiscuity.

#83 - UNCOVERING THE FUNCTION OF ADP-RIBOSYLATION WITH COVALENT PARP INHIBITORS

Daniel S. Bejan, Oregon Health & Science University
Jonathan Pruneda, Oregon Health & Science University

Michael S. Cohen, Oregon Health & Science University

ADP-ribosylation is a critical post-translational modification carried out by a family of 17 enzymes in humans known as PARPs. Most of the PARP inhibitors developed to date target PARP1, an important first responder of DNA damage and a well-validated cancer target. Other

PARP members are also becoming implicated in diseases such as cancer and inflammation, however, the role of ADP-ribosylation from these PARPs is far less understood, in large part due to the lack of appropriately selective inhibitors. Developing selective inhibitors for PARPs is challenging due to the highly conserved NAD⁺-binding site shared between family members. To overcome this challenge, we identify and exploit non-conserved cysteines near the active site of PARPs for covalently targeting with appropriately positioned electrophiles. This strategy has resulted in the first example of cysteine-targeted covalent inhibitors for two PARP members. The first is PARP16, an emerging novel therapeutic target in ovarian cancer. We developed DB008, an acrylamide-containing inhibitor that selectively targets Cys169 of PARP16 and discovered that DB008 prevents PARP16 auto-aggregation upon nutrient stress, suggesting that catalytic activity regulates PARP16 solubility in cells. The second is PARP10, an enigmatic enzyme implicated in cancer, innate immune signaling, and neurodegeneration. We developed a novel fluorescent PARP10 covalent inhibitor and discovered a crosstalk between PARP10-mediated ADP-ribosylation and ubiquitination. In summary, we have developed first-in-class covalent probes that will serve as useful tools to uncover the physiological and pathophysiological roles of ADP-ribosylation in understudied PARP members.

#17 - DEVELOPING A METHOD FOR PROTEIN EDITING IN MAMMALIAN CELLS

Ms. Jenna Beyer, University of Pennsylvania

Dr. Jay Serebrenik, University of Pennsylvania

Dr. Ophir Shalem, University of Pennsylvania

Dr. George Burslem, University of Pennsylvania

The ability to tag proteins has been transformative for the study of proteins, and has enabled researchers to track a protein of interest's location, functions, and interactions. However, adding a tag to a protein comes with

its own set of problems, particularly in mammalian cells. All tags, whether a 30 kDa GFP or a short epitope tag, are adding additional residues and subsequent bulk to the tagged protein, and this addition can impact key characteristics of the protein of interest, such as protein localization, half-life, activity, or interactions. For example, it is known that some proteins cannot tolerate a tag at their N or C termini, requiring their unaltered, native sequence to carry out specific functions. In a similar vein, many proteins lack specific antibodies, rendering them challenging to study. From these scenarios, it is clear that there is a need for a less disruptive tagging strategy that still enables informative experiments. To fill this gap in the field, we have developed a method for protein editing that enables the rapid installation of unnatural amino acids into proteins in mammalian cells. In this way, we can introduce useful functional groups with temporal resolution, in an effort to carry out classic cell biology experiments with significantly less disruption to the protein of interest. In order to do this, our technology combines split intein-mediated protein trans-splicing and genetic code expansion to "edit" an unnatural amino acid, such as a click chemistry handle, photo-crosslinker, or authentic PTM, into a user-defined site in proteins inside mammalian cells. To demonstrate the utility of this technology, we have installed the unnatural amino acid p-azido-phenylalanine (pAzF), as well as TAMRA and biotin conjugated by click chemistry to pAzF, into several proteins in mammalian cells, including calnexin and β -actin. Using these proteins as model systems, we have been able to validate our protein editing method by microscopy, mass spectrometry, and immunoblotting. We demonstrate that our protein editing method is rapid, nearly traceless, and can be easily multiplexed to incorporate a variety of useful labels. We next plan to extend our technology to other unnatural amino acids, including authentic PTMs, and to begin applying our technology towards interesting biological problems in mammalian cells.

#97 - GENETICALLY ENGINEERED LACTIPLANTIBACILLUS PLANTARUM AS BIOSENSOR PROBES FOR THE LUNGS

Dr. Michael Brasino, Oregon Health & Science University

Mr. Eli Wagnell, Oregon Health & Science University

Mr. Sam Drenan, Oregon Health & Science University

Ms. Elise Manalo-Hall, Oregon Health & Science University

Prof. Jared Fischer, Oregon Health & Science University

Prof. Justin Merritt, Oregon Health & Science University

My team and I are developing a platform biosensor technology comprising genetically engineered *Lactiplantibacillus plantarum* administered to the lower respiratory tract. Using mouse models, we have demonstrated that these engineered bacteria may be specifically delivered to the lungs, where they do not colonize or produce a significant adaptive immune response. Engineered bacteria can respond to molecules detected in the lungs by secreting a synthetic biomarker detectable in mouse urine. They can also secrete synthetic biomarkers which are easily detected with off-the-shelf at-home tests. We will present recent results using these engineered bacteria to detect model lung tumors in immune-competent mice and ongoing efforts to engineer bacterial receptors for specific tumor-secreted proteins.

#37 - CRYO-EM STUDY OF GLYCOSOME ISOLATED FROM LEISHMANIA TARENTOLAE

Dr. Jianhao Cao, Oregon Health & Science University

Dr. Phil Yates, Oregon Health & Science University

Dr. Liman Zhang, Oregon Health & Science University

Trypanosomatida contains a group of kinetoplastid unicellular eukaryotes having a well-defined nucleus and other cell organelles including glycosome, kinetoplasts and flagella. Some species have life-cycles involving both human and other vertebrate or invertebrate hosts. Various species of *Leishmania* in the

Trypanosomatida genus typically transmitted by sandflies and cause series of clinical manifestations named Leishmaniasis. The glycosome is a featured membrane-enclosed organelle in the body of *Leishmania*. It is known to harbor most peroxisomal enzymes and 9 glycolytic enzymes for energy metabolism. In the present study, we isolate glycosome from *Leishmania tarentolae* and use both negative-stain and cryo-EM to confirm the pure glycosome fraction and identify the glycosome fraction with featured protein arginase (ARG). In future work, we can use both single particle analysis and tomography to study the architecture of both the glycosome organelle and proteins/complexes which helps to explore new drug targets.

#21 - HUMANIZED PROTEIN SPONGES- A NEW APPROACH FOR OPIOID OVERDOSE TREATMENT

Ishita Chandra, Michigan State University

Courtney Bingham, Michigan State University

Mona Babak, Michigan State University

Prof. Kenneth Merz, Michigan State University

Prof. James Geiger, Michigan State University

Prof. Babak Borhan, Michigan State University

The misuse and addiction of opioids such as fentanyl is a serious national crisis. In most cases, the cause of death is respiratory suppression. Presently, naloxone is the drug of choice, which competitively binds μ receptors and acts as an antagonist. However, its receptor binding half-life is significantly shorter than that of opioids. In our lab, we are designing 'humanized' proteins that can bind fentanyl with high affinity and slow off-rates to assist with removal of opioids from the system upon overdose to prevent relapse. The process of protein design and optimization of opioid binding will be presented.

**#65 - COUPLED FLASH CHROMATOGRAPHY-
MASS SPECTROMETRY TOOLS IN THE
MEDICINAL CHEMISTRY CORE**

Dr. Jordan Devereaux, Oregon Health & Science University

Dr. Victoria Halls, Oregon Health & Science University

Dr. Tapasree Banerji, Oregon Health & Science University

Dr. Aaron Nilsen, Oregon Health & Science University

The OHSU Med Chem Core has a new Biotage Isolera coupled to our Advion compact mass spectrometer, creating an instrument that is capable of preparative normal or reverse phase flash-MS. Have you ever wanted to run real time mass spec while simultaneously performing a separation? Have you ever wanted to know the mass spec profile of each of your fractions? Have you ever wanted to collect fractions that contain only a specified mass? Using our new prep flash-MS, now you can optimize your workflow and turn an all-day separation process into something that can be done in under an hour. If you would like to learn more, please visit us at our poster.

**#23 - MAPPING MOLECULAR INTERACTIONS
WITH THE PROTEIN PHOSPHATASE 5 (PP5)
IDENTIFIES NEW POSSIBILITIES FOR CHEMICAL
PROBES**

Dr. Shweta Devi, University of California San Francisco

Annie Charvat, University of California San Francisco

Prof. Jason Gestwicki, University of California San Francisco

Protein quality control (PQC) involves a network of proteins that work together to maintain protein homeostasis. The molecular chaperones, Hsp70 and Hsp90, are primary components of the PQC system, wherein they coordinate the activity of partners, including the protein phosphatase, PP5. Unlike other phosphatases, PP5 does not utilize a regulatory subunit; rather, the chaperones act as adapters to bring the enzyme into proximity with substrates. The

protein-protein interactions (PPIs) between Hsp70/Hsp90 and PP5 is known to require the EEVD motif that is located at the C-termini of the chaperones and the tetratricopeptide (TPR) domain within the phosphatase. Yet, the molecular determinants of these PPIs are not known. To gain insight into this question, we screened a library of ~640,000 peptides for binding to PP5's TPR domain using a fluorescent polarization (FP) assay. The optimal sequence, WDDVD, from this screen bound ~4-fold better than the natural sequence and an alanine scan identified the key residues. Finally, binding to the chaperones is known to activate PP5's enzyme activity. Using our library of EEVD-like peptides, we found that affinity is the primary determinant of allosteric activation, a result further supported by next generation differential scanning fluorimetry (DSF) experiments. Together, these data identify the determinants of PP5 binding and provide a foundation for building chemical probes that bind/activate PP5.

**#61 - VERSATILE INTERACTING PEPTIDE
TAGS: AN EXPANDED TOOLKIT FOR
MULTICOLOR MICROSCOPY**

Dr. Kaylyn Devlin, Oregon Health & Science University

Ms. Alexa Suyama, Oregon Health & Science University

Dr. Miguel Macias-Contreras, Oregon Health & Science University

Dr. Julia K. Doh, Oregon Health & Science University

Dr. Ujwal Shinde, Oregon Health & Science University

Dr. Kimberly E. Beatty, Oregon Health & Science University

Genetic tags are transformative tools for investigating the function, localization, and interactions of cellular proteins. Single protein imaging and analysis has been enabled by fusion to a protein tag, such as green fluorescent protein, HaloTag, or SNAP-Tag. However, the large size of these tags (~30 kDa) can change a protein's function, localization, and binding interactions. We developed versatile interacting

peptide (VIP) tags for when a small tag (4.3 kDa) is preferred. VIP tags enable observations of protein localization and trafficking with bright fluorophores or nanoparticles. Labeling is mediated by the formation of a high affinity, biocompatible heterodimeric coiled coil between a genetically-encoded tag and an exogenously added reporter-labeled peptide. We have used VIP tags for dynamic imaging, correlative light and electron microscopy, and protein translocation. In our most recent work, we created a set of three tags for the selective labeling of more than one protein at once: MiniVIPER, TinyVIPER, and PunyVIPER. This trio of tags can be used to obtain comprehensive information on a protein's behavior in situ. Labeling via each tag is self-sorting and highly specific. The orthogonality of the tags enables simultaneous labeling of up to three protein targets. This poster will describe the design and validation of these tags in both live and fixed cells, illustrating how VIP tags are versatile, advantageous tools for multicolor microscopy.

#63 - REGULATION OF THE JAK-STAT PATHWAY IN DROSOPHILA BY PARASITOID WASP VENOM

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 Carrie Marean-Reardon, Oregon State University
 Dr. Nathan T. Mortimer, Oregon State University

Each year an overwhelming amount of people (approximately 60,000) are diagnosed with leukemia. Leukemia is a cancer that affects the differentiation of the hematopoietic stem cells (HSC). The JAK-STAT pathway regulates the production and replenishment of HSCs through cell mediated response to cytokines and growth factors. Gain of function mutations in this pathway are common drivers in the development of leukemia. These mutations cause unregulated transcription of the Stat target genes, leading to proliferation, progression, and survival of the cancer cells. *Drosophila melanogaster*, a model organism due to its simplified system, possess a homologous JAK-STAT pathway to that of

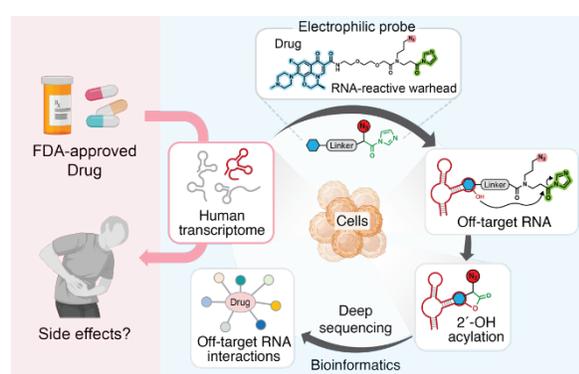
humans. When a gain of function mutation is introduced into the *Drosophila* JAK homolog hop[Tum], a leukemia-like phenotype is exhibited. We have shown that venom from the parasitoid wasp species *Ganaspis xanthopoda* inhibits the JAK-STAT pathway, and thus, the characteristic leukemia phenotype is not observed. Currently it is unknown what component of the wasp venom is inhibiting the pathway. In an effort to identify the protein component responsible for this inhibition, we have generated a fly line that expresses GFP in response to JAK-STAT signaling, and links GFP fluorescence intensity to the activity of the JAK-STAT pathway. Through the use of larval immune cell fluorescence assays, we have determined the baseline for fluorescence intensity that indicates the JAK-STAT pathway is active. To identify the active protein component in the venom, we will subject the wasp venom to fractionation via a sucrose gradient and will then inject the fractions into the larvae. Using the immune cell fluorescence assay, we can then determine which fraction contains the active protein, which allows us to narrow the identification of the protein. Due to the prevalence of leukemia, identifying novel JAK-STAT regulators is an important research goal, as a better understanding of the pathway and its regulation can pave the way for potential therapeutics.

#11 - PERVASIVE TRANSCRIPTOME-INTERACTION OF PROTEIN-TARGETED DRUGS

Dr. Linglan Fang, Stanford University
 Dr. Willem A. Velema, Stanford University
 Dr. Yujeong Lee, Stanford University
 Dr. Lu Xiao, Stanford University
 Dr. Michael G. Mohsen, Stanford University
 Dr. Anna M. Kietrys, Stanford University
 Prof. Eric T. Kool, Stanford University

The off-target toxicity of drugs targeted to proteins imparts substantial health and economic

costs. Proteome interaction studies can reveal off-target effects with unintended proteins; however, little attention has been paid to intracellular RNAs as potential off targets that may contribute to toxicity. To begin to assess this, we developed a reactivity-based RNA profiling (RBRP) methodology, and applied it to uncover transcriptome interactions of a set of FDA-approved small-molecule drugs in vivo. We show that these protein-targeted drugs pervasively interact with the human transcriptome and can exert unintended biological effects on RNA function. In addition, we show that many off-target interactions occur at RNA loci associated with protein binding and structural changes, allowing us to generate hypotheses to infer the biological consequences of RNA off-target binding. The results suggest that rigorous characterization of drugs' transcriptome interactions may help assess target specificity and potentially avoid toxicity and clinical failures.



#19 - MULTI-FUNCTIONALIZED LIPID DERIVATIVES AS TOOLS TO STUDY VIRAL INFECTIONS

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Dr. Frank Stein, European Molecular Biology Laboratory, Heidelberg

Dr. Per Haberkant, European Molecular Biology Laboratory, Heidelberg

Dr. Fikadu Tafesse, Oregon Health & Science University

Dr. Carsten Schultz, Oregon Health & Science University

Positive-stranded RNA viruses, including flaviviruses and coronaviruses, produce dramatic membrane rearrangements within their host cells. Both SARS-CoV-2 and ZIKV produce a characteristic array of double-membraned vesicles, thought to be the site of viral replication, and convoluted membranes, with less well-defined functions. We have previously shown that both ZIKV and SARS-CoV-2 exert dramatic changes on the host lipidome, notably inducing high levels of ceramide (ZIKV) and dihydroceramide (SARS-CoV-2). To interrogate a functional link between sphingolipids and viral life cycles, we synthesized trifunctional sphingosine (TF-Sph), trifunctional sphinganine (TF-Spa), and a control fatty acid (TF-FA), to investigate how these global changes correspond to subcellular localization and protein-lipid binding in both viruses. We found that sphingoid bases have distinct relationships with centers of viral replication 24 hours after infection with either ZIKV or SARS-CoV-2. We further observed direct, unique interactions between individual sphingoid bases and a handful of viral proteins, as well as changed interactions with many host proteins, including proteins involved in membrane transport, cytoskeletal organization, and cell distress pathways. These results both demonstrate the power of functionalized lipids to explore the function of individual molecules in complicated systems, and begin to reveal the complex ways in which viral infect can perturb the distribution and interaction network of particular lipids.

#79 - DISCOVERY OF PEPTIDE-MHC COMPLEXES FOR TARGETED CANCER IMMUNOTHERAPY

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Dr. Ines B. Folger, University of California, San Francisco

Prof. James A. Wells, University of California, San Francisco

Immunotherapy is a promising strategy for targeted elimination of cancer cells in patients. A class of cell surface proteins called major histocompatibility complexes (MHCs) has recently shown great promise as targets that are useful for cancer immunotherapy. MHCs are key to immunosurveillance, presenting thousands of short, proteolyzed peptides derived from intracellular proteins on the cell surface.

Cancerous cells often display a distinct collection of peptides on MHCs and thus represent a rich source of novel cancer biomarkers useful for development of novel immunotherapeutics. Nevertheless, the identification of peptide-MHC complexes that are shared among many different cancer patients remains highly challenging. Here, we identify MHC complexes that correlate to common events in carcinogenesis, such as hyperactive oncoproteins, and therefore have the potential to be more “publicly” presented across cancer patients. We further developed antibodies that can specifically target oncogene-induced MHC complexes, which demonstrates their therapeutic potential. In summary, our approach holds promise to expedite therapeutic discovery for targeted immunotherapy.

#101 - DEVELOPMENT OF ^{19}F MRI PROBE USING LIPID NANODISC

Mr. Kodai Fukushima, Graduate School of Engineering, Osaka University

Dr. Tomoya Yamamoto, Graduate School of Engineering, Osaka University and Immunology Frontier Research Center, Osaka University

Prof. Kazuya Kikushi, Graduate School of Engineering, Osaka University and Immunology Frontier Research Center, Osaka University

Magnetic resonance imaging (MRI) is a useful technique for non-invasive visualization of deep tissues *in vivo*. In particular, ^{19}F MRI, which visualizes the location of probes containing ^{19}F nuclei, has recently attracted attention. Unlike ^1H MRI, ^{19}F MRI has low intrinsic background signals. Because of these advantages, nanoparticles containing ^{19}F nuclei, which are designed to accumulate in the specific tissues

and organs such as tumor cell, have been developed as ^{19}F MRI probes. Previously, various types of ^{19}F MRI probes have been developed such as emulsion-based particles^[1], silica-coated nanoparticles^[2], and so on. However, these ^{19}F MRI probes have problems with particle size control, *in vivo* toxicity, and surface modification methods.

To solve these problems, we came up with the application of lipid nanodiscs for ^{19}F MRI probes. Lipid nanodiscs are monodisperse particles 10-20 nm in size, consisting of a phospholipid bilayer surrounded by a ring of amphiphilic polymers or proteins. The size of nanodiscs is easily controlled by the length and equivalent of the amphiphilic molecules. In addition, lipid nanodiscs are expected to be less toxic than previously developed ^{19}F MRI probes due to the absence of inorganic materials. Furthermore, surface modification can be easily conducted by mixing the phospholipids with functional substituents during the preparation of nanodiscs. Based on these points, we aim to introduce ^{19}F atoms into lipid nanodiscs and use them for ^{19}F MRI probes.

For this purpose, we synthesized two types of ^{19}F -labeled phospholipids, in which ^{19}F atoms are introduced in the head group or acyl chains. These structures were designed based on structure of phosphatidylcholine to obtain their motility and stability in phospholipid bilayers. We prepared ^{19}F -labeled nanodiscs using ^{19}F -labeled phospholipids and established the purification methods. Transmission electron microscope imaging showed the formation of particles with the size of 10-20 nm. The long T_2 relaxation time of ^{19}F -labeled nanodiscs demonstrated the potential applicability for ^{19}F MRI measurements. Finally, ^{19}F MR signals from ^{19}F -labeled nanodiscs were also observed in ^{19}F MRI measurements.

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[2] H. Matsushita *et al.*, *Angew. Chem. Int. Ed.* **2014**, *53*, 1008.

#7 - PHOTOSWITCHABLE TOOLS TO OPTICALLY CONTROL CB1 SIGNALING IN NEURONAL CIRCUITRY

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Dr. Roman C. Sarrott, ETH Zürich

Dr. Patrick Pfaff, ETH Zürich

Miroslav Kosar, ETH Zürich

Dr. Erick Carreira, ETH Zürich

Dr. James Frank, Oregon Health & Science University

Marijuana consumption can stimulate appetite and the pleasurable impact of food through its psychoactive component, D⁹ - tetrahydrocannabinol (THC), which targets the CB1 cannabinoid receptor. CB1's role in the brain's reward circuitry is largely unexplored due to limitations in tools that can manipulate endogenous receptor activity with spatiotemporal acuity. Therefore, we developed photoswitchable analogs of THC that optically control endogenous CB1 activity. One of these analogs, *azo*-THC, is a THC aromatic core bound to an azobenzene that switches the compound from a least to more active state under different light conditions. The other THC analog is structurally similar and switches under the same light conditions but it is also membraned anchored, thus it provides both spatial and temporal acuity. We demonstrate that these tools help us interrogate the endocannabinoid system in a receptor-specific manner *in vitro*. We later plan to use *azo*-THC *in vivo* to interrogate CB1's role in mediating a sugar reward circuit from the ventral hippocampus to the nucleus accumbens shell. This work will advance the use of optically activated ligands to study endocannabinoid signaling with improved spatiotemporal precision.

#49 - EXPLORING PT(II)-INDUCED NUCLEOLAR STRESS THROUGH "CLICK CHEMISTRY"

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Paul D. O'Dowd, RCSI, Dublin, Ireland; SSPC, the Science Foundation Ireland Research Centre for Pharmaceuticals, Ireland

Hannah C. Pigg, University of Oregon

Katelyn R. Alley, University of Oregon

Prof. Darren M. Griffith, RCSI, Dublin, Ireland; SSPC, the Science Foundation Ireland Research Centre for Pharmaceuticals, Ireland

Prof. Victoria J. DeRose, University of Oregon

Platinum(II) chemotherapeutic complexes have been utilized extensively in the clinic as anticancer drugs for nearly fifty years. Currently, there are three FDA-approved chemotherapeutic Pt(II) complexes: cisplatin, carboplatin, and oxaliplatin. Until recently, all three of these compounds were thought to induce cellular apoptosis through the DNA damage response pathway. Studies within the last decade, however, suggest that oxaliplatin may instead induce cell death through an alternative nucleolar stress pathway. Nucleolar stress induction by small molecules is not well understood, and further investigation of this pathway may provide both basic knowledge about nucleolar stress and insight for the development of more tunable Pt(II) chemotherapeutics. Through a previous structure-function analysis, it was determined that nucleolar stress induction is highly sensitive to modifications at the 4-position of the 1,2-diaminocyclohexane (DACH) ring of oxaliplatin.¹ Specifically, less-rigid substituents (methyl, ethyl, propyl) induce nucleolar stress, while more rigid and bulkier substituents (isopropyl, acetamide) do not. Informed by these findings, a novel click-capable oxaliplatin mimic, (*cis*-[(1R,2R,4S) 4-methylazido-1,2-cyclohexanediamine]dichlorido platinum(II)), was synthesized and identified to induce nucleolar stress through NPM1 relocalization, fibrillarin redistribution, and H2AX phosphorylation assays.² This complex provides

a new tool to localize and identify cellular targets of Pt(II) nucleolar stress inducers through post-treatment Cu-catalyzed azide-alkyne cycloaddition, and may help to better understand Pt(II)-induced nucleolar stress. Additional work has focused on installing click-capable handles onto 1,2-diaminocyclopentane (DACP) of DACP-Pt, and 1,2-phenylenediamine (OPDA) of OPDA-Pt, both of which have been previously identified as nucleolar stress inducers.^{3,4} Click-capable DACP-Pt is a close derivative of the previously synthesized click-capable oxaliplatin complex, but preliminary results suggest that the extent of nucleolar stress induced by this compound may be different. The unique structure and electronics of OPDA-Pt position it as a potential novel tool to study nucleolar stress induction.

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#75 - SPATIOTEMPORAL CONTROL OF ANTIBODY ACTIVITY USING A MODULAR PROTEIN-L TETHERED BLOCKING PEPTIDE

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Dr. Michael Brasino, Oregon Health & Science University

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Dr. Sean Speese, Oregon Health & Science University

Mr. Eli Wagnell, Oregon Health & Science University

Prof. Stuart D. Ibsen, Oregon Health & Science University

Monoclonal antibodies (mAbs) have revolutionized cancer therapy by targeting specific cell surface antigens with remarkable precision. However, their inherent "on-state" binding often leads to unforeseen adverse side effects including runaway inflammation, limiting their therapeutic potential.¹ Conventional approaches to mitigate these effects involve intricate antibody re-design², which is limited by high cost and a low throughput return. To address this limitation, we developed a peptide blocker with a modular design to control mAb binding activity without the need for antibody re-design. Our approach involves the design of a new blocking construct that utilizes both covalent and non-covalent interactions with the antibody. At one end of the construct there is a blocking peptide designed to interact with the antibody binding site. We use a flexible tether to attach the blocking peptide to an engineered protein L (PpL) based anchor.³ Covalent photo-triggered crosslinking enabled successful and reproducible PpL conjugation at the antibody light chain interface allowing the tethered blocking peptide to bind the active site of the antibody. This blocking can be selectively reversed through enzymatic or light-based cleavage of the tether, enabling precise spatiotemporal control over antibody activity. Through modified column chromatography purification methods, unbound protein L was removed from the reaction mixture, resulting in blocked antibodies that showed a 25-fold reduction in binding activity. Moreover, the application of this activatable antibody design in A431 (EGFR +) cell culture revealed a significant block and restoration of antibody binding activity. These advancements underscore the potential of our approach for future modular control over commercial and therapeutic antibody-based applications.

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#1 - MUTATIONAL ANALYSIS OF DNAJC13, AN UNCHARACTERIZED ENDOSOMAL CONSTITUENT

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Ms. Emily Holland, Oregon Health & Science University

Ms. Emily Hutchinson, Oregon Health & Science University

Dr. Braden Lobingier, Oregon Health & Science University

Initiation of physiologic responses to external stimuli hinges on functional membrane trafficking by endosomes. However, functional consequences of many endosomal proteins have not been characterized. One such endosome resident is DNAJC13, a large J Domain protein. This protein possesses N-terminal, PH-like domains that confer affinity with phosphoinositol-3-phosphate (PI3P) (1), providing a means by which DNAJC13 could commute to PI3P-rich, endosomal membranes. At the obverse terminus of DNAJC13, there is what AlphaFold prediction suggests is an unstructured, potentially-flexible c-terminal "tail" (2,3,4), perhaps exposing a control point for DNAJC13 function. Upon truncation of the c-terminus, we observed markedly different localization of DNAJC13 in HEK293 cells. Namely, the full-length protein is diffusely distributed in cytosol, and the truncated protein is primarily seen at membranes of large, perinuclear, luminal vesicles in HEK293 cells. These vesicles seem to include endosomal markers including Rab5 and FYVE. Our observations could reveal a regulatory mechanism for endosomal targeting of DNAJC13 involving interplay between its N-terminal PH-like domains and its C-terminus. Puzzlingly, localization of the protein in other cell types dramatically differs from the localization we observe in HEK293 cells. Namely, in HeLa cells, we were able to corroborate earlier observations of DNAJC13 localization on puncta and in cytosol—not on large, perinuclear vesicles (5).

Prior work involving DNAJC13 localization effects demonstrates that mutations to a PH-like domain of DNAJC13 result in diffuse, cytosolic localization in HeLa cells, similar to localization we observe for the wild-type protein in HEK293 cells (1). To test whether C-terminal mutations improve the affinity of DNAJC13 with PI3Ps conferring noticeably improved localization to PI3P-rich membranes in HEK293 cells, we tested the affinity of wild-type and c-terminally-mutated DNAJC13 with PI3P beads. This work demonstrates an additive affinity of c-terminally mutated DNAJC13 with PI3P, perhaps suggesting regulation of the N-terminal PH-like domains by its C-terminus of DNAJC13.

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#3 - MECHANISTIC STUDIES OF CYSTEINE ARYLATION WITH PYRIDINIUM SALTS

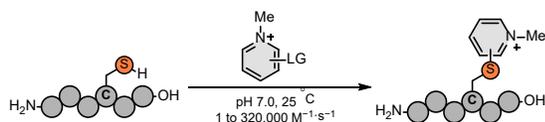
Mr. Daniel S. Honeycutt, University of Rhode Island

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Prof. Fang Wang, University of Rhode Island

Chemical modification of cysteine is among the most commonly employed strategies for bioconjugation. Here, we present a library of simple pyridinium salts exhibiting remarkable reactivity toward cysteine. This methodology enables rapid metal-free, catalyst-free cysteine arylation under mild conditions. Comprehensive

kinetic studies, along with other investigations, have been conducted to quantify the reactivity of these pyridinium-based reagents and reveal the mechanistic basis of this unusually fast chemistry. Such information facilitates the rational design of versatile and practical synthetic tools for the construction of structurally diverse constructs via cysteine functionalization.



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#31 - LIPIDS AND FLAVIVIRUSES - A CLOSER LOOK INTO PHOSPHATIDYLINOSITOL AND ITS DERIVATIVES

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Viral pathogens exploit host cellular machineries for their propagation and lead to extensive remodeling of cellular membranes to allow efficient replication while evading cellular immune responses. Recent studies have highlighted specific lipid species as critical regulators of virus entrance, replication, assembly, and egress. Our research and that of others have shown that sphingolipids and phospholipids, like phosphatidylinositol, play a role in the propagation of (+)-sense RNA viruses, with a time-relevant focus on Flaviviruses. A precise molecular mechanism by which these lipids change the virus life cycle remains elusive

due to the number of variables involved, i.e. lipid species, localization, and protein interactions. To gain a more thorough understanding of the exact mechanism by which lipids, particularly phosphatidylinositol (PI) and its derivatives (PIP), regulate Flavivirus propagation, we are combining virology, biochemistry, genetics, and imaging techniques with highly functionalized lipid tools. Specifically, we first examine the impact of the virus on PI metabolism using specialized Mass Spectrometry, then determine how specific PI/PIP species influence the virus life cycle. Furthermore, short-lived changes in lipid metabolism, localization, and protein interaction during virus infection will be determined using highly functional lipid probes. These investigations will allow us to formulate the molecular mechanisms behind PI's influence on the virus-host interactions. Moreover, we will gain a better understanding of how manipulating PI/PIP levels can be used to influence viral entry, modulate the host immune response, and disrupt viral assembly and release. Expanding our knowledge of lipid-mediated mechanisms in virus propagation holds great potential for combating viral diseases effectively.

#47 - O-GLCNACYLATION OF HSP27 PROMOTES BETTER SUBSTRATE HANDOFF BY BAG3

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HSP27 is a chaperone protein involved in maintaining protein homeostasis in humans by keeping unfolded or misfolded proteins soluble ("holdase" function). Under conditions of cellular stress, expression of HSPs is upregulated. Cellular stress also increases levels of protein O-GlcNAc modification. O-GlcNAc is an

intracellular post-translational modification that is misregulated in neurodegenerative diseases. O-GlcNAcylation near the C-terminal IPV motif of HSP27 prevents its interaction with the central α -crystallin domain (ACD). This opens up the ACD to bind more client amyloidogenic proteins.

HSP27 also functions as part of a greater heat shock system with ATP-dependent chaperones like HSP70, which actively refolds misfolded proteins ("foldase" function). BAG3 is a scaffolding protein that bridges "holdases" such as HSP27 and "foldases" like HSP70 to form a ternary complex. Given that BAG3 binds to the ACD of HSP27, we hypothesized that O-GlcNAcylation of HSP27 would promote its interaction with BAG3 and facilitate communication with HSP70.

Here, we used protein semi-synthesis to generate O-GlcNAc modified HSP27 in order to assess its differential protein-protein interactions through *in vitro* biochemistry. O-GlcNAc modified HSP27 shows increased interaction with BAG3, which promotes protein refolding by the HSP27-BAG3-HSP70 complex. After characterizing this interaction, we identified other protein interactions that were regulated by O-GlcNAc. We validated this proteomics data by studying CRYAB, another small heat shock protein. CRYAB was found to prefer forming heterooligomers with unmodified HSP27 over O-GlcNAcylated HSP27. Our results provide insight into O-GlcNAc's multifaceted role in preventing and resolving protein misfolding and aggregation.

#15 - THE PREPARATION OF HYDROPHILIC POLYCHROMATIC EMISSIVE CARBON DOTS AS SUPERIOR BIOIMAGING AND NIR-RESPONSIVE PHOTOTHERMAL BACTERICIDAL AGENT

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Prof. Jongsung Kim, Gachon University

Highly photoluminescent greenish-yellow emissive CDs have been prepared based on

nitration followed by hydrothermal carbonization of the polycyclic aromatic hydrocarbon precursor, perylene. The perylene-derived CDs (PY-CDs) exhibited an excellent NIR-light (808 nm) harvesting property toward high photothermal conversion efficiency (PCE = ~56.7%) and thus demonstrated remarkable NIR-light responsive photothermal bactericidal performance. Furthermore, these fluorescent PY-CD nanoprobes displayed excitation-dependent polychromatic emissions in the range of 538–600 nm, with the maximum emission at 538 nm. This enables intense multicolor biological imaging of cellular substances with long-term photostability, nontoxicity, and effective subcellular distribution. Our investigation reveals that the bactericidal action of PY-CDs is likely due to the elevated ROS amplification in cooperation with the hyperthermia effect. This study offers a potential substitute for multi color imaging-guided metal-free carbon-based photo thermal therapy.

#53 - ASSESSING THE LIGAND SPECIFICITY OF NAIP BY STRUCTURAL STUDIES

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Dr. Jianhao Cao, Oregon Health & Science University

Dr. Liman Zhang, Oregon Health & Science University

The classical NAIP/NLRC4 inflammasome pathway is activated when the NAIP protein (NLR family apoptosis inhibitory protein) recognizes bacterial ligand proteins. Humans have only one NAIP, commonly called hNAIP, that recognizes the T3SS (type 3 secretion system) needle protein, while mice have multiple NAIPs that each interact with a specific bacterial ligand. In the past our lab has looked at the ability of mouse NAIP5 in recognizing bacterial flagella, however, the scientific community has not reached a consensus on whether hNAIP is able to even recognize Flagella. To test a more relevant protein to humans we started looking into mouse NAIP1 which, similar to the hNAIP, are known to interact with T3SS needle protein. The structure of NAIP1 is currently unknown so

our goal is to use cryo-EM to solve the structure of the activated NAIP1. By testing binding conditions with multiple needle proteins, we obtained a stable NAIP1-ligand complex and optimized purification for cryo-EM screening and data collection. We believe this data will provide a better understanding of bacterial infection response in humans and the mechanism of protein recognition between immune receptors and PAMPs (pathogen-associated molecular patterns).

#93 - PROFILING ORGANOPHOSPHATE PESTICIDE PROTEIN TARGETS USING ACTIVITY-BASED PROBES

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Dr. Mark Maupin, Pacific Northwest National Laboratory

Dr. Samantha M. Powell, Pacific Northwest National Laboratory

Ms. Jennifer Walker, Pacific Northwest National Laboratory

Mr. Gerard X. Lomas, Pacific Northwest National Laboratory

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Dr. William C. Nelson, Pacific Northwest National Laboratory

Dr. Stephen J. Callister, Pacific Northwest National Laboratory

Dr. Heather A. Colburn, Pacific Northwest National Laboratory

Dr. Jordan N. Smith, Pacific Northwest National Laboratory

Dr. John R. Cort, Pacific Northwest National Laboratory

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Organophosphates (OPs) are highly toxic chemicals that are used worldwide as pesticides.

Acute exposures to OP pesticides through inhalation, ingestion, or dermal contact can lead to muscle weakness, paralysis, and seizure due to cholinesterase inhibition. Long-term neurological impacts of OP exposures, which may be mediated by non-cholinergic mechanisms, are a growing area of interest in understanding OP toxicity. We applied OP activity-based probes (ABPs) for activity-based protein profiling (ABPP) to identifying changes in enzymatic activities after in vitro exposure to paraoxon, a model OP pesticide, across 5 tissue types from 6 mammalian species, including humans. We identified many proteins known to be inhibited by OPs and characterized their distribution in specific tissues and animal models. We also identified proteins not previously described as OP targets, including endoplasmic reticulum chaperone (ENPL), calreticulin (CALR), and Parkinson's disease protein (PARK7). Molecular docking for ENPL showed a possible binding pocket for paraoxon. We are now experimentally assessing the effects of OP binding on protein functions for these targets identified through our ABPP approach. This work will help clarify the effects of OP toxicity beyond cholinesterase inhibition and help identify key proteins or pathways for therapeutic development to treat OP exposures. specific tissues and animal models. We also identified proteins not previously described as OP targets, including endoplasmic reticulum chaperone (ENPL), calreticulin (CALR), and Parkinson's disease protein (PARK7). Molecular docking for ENPL showed a possible binding pocket for paraoxon. We are now experimentally assessing the effects of OP binding on protein functions for these targets identified through our ABPP approach. This work will help clarify the effects of OP toxicity beyond cholinesterase inhibition and help identify key proteins or pathways for therapeutic development to treat OP exposures.

#43 - NEW INSIGHTS INTO THE ROLE OF LIPOPOLYSACCHARIDES IN ACTIVATING THE COAGULATION CONTACT SYSTEM

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Dr. Cristina Puy, Oregon Health & Science University

Prof. Joseph Aslan, Oregon Health & Science University

Prof. Owen McCarty, Oregon Health & Science University

Background: Lipopolysaccharides (LPS) released into the bloodstream during infection are known to dysregulate blood coagulation. Since LPS form negatively charged aggregates in aqueous environments, they may initiate blood coagulation through activation of the contact system. However, little is known about the role of LPS in the interactions with contact system proteins.

Aims: To investigate the mechanisms underlying the interaction and activation of FXII by distinct LPS chemotypes.

Methods: LPS aggregates – formed by either smooth (O111:B4), semi-rough (O26:B6), and rough (Rd2) chemotypes of *E. coli* LPS – were analyzed by dynamic light scattering and zeta potential (ZP). Binding of FXII to LPS was analyzed by fluorescence spectroscopy. Enzymatic assays were employed to study the effects of LPS on FXII activation.

Results: O111:B4, O26:B6, and Rd2 formed aggregates with sizes of 101, 196, and 211 nm, respectively. In the absence of NaCl, the aggregates showed a ZP of -2.2, -24.5, and -55.4 mV. However, in 150 mM NaCl, the ZP were -0.51, -13.8, and -8 mV. As salt concentration is increased, more cations adsorb to the negatively charged LPS, neutralizing the surface charge. Using fluorescence spectroscopy, we demonstrate that all LPS in aggregate forms bind to FXII with similar affinity ($K_D \sim 0.3 \mu\text{M}$). However, the interaction with O26:B6 induces allosteric changes to the protein global structure,

exposing the active site of the zymogen and promoting the formation of a single-chain FXII_{act}. The complex O26:B6– FXII_{act} stimulate the activation of prekallikrein and FXI, subsequently leading to generation of kallikrein and FXIa.

Conclusions: Distinct chemotypes of LPS appear to have differential impact on the interaction and activation of FXII according to the polysaccharide physicochemical properties, showing activity towards physiological substrates, which may represent the primary event in initiating contact system activation *in vivo*.

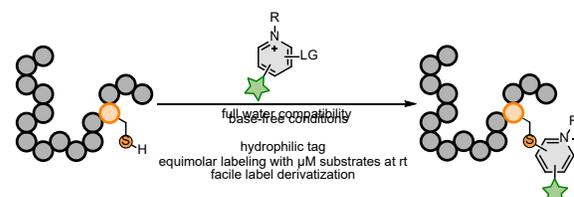
#5 - ULTRA-RAPID ELECTROPHILIC CYSTEINE ARYLATION

Mr. Bradley M. Lipka, University of Rhode Island

Dr. Jacob M. Goldberg, Colgate University

Dr. Fang Wang, University of Rhode Island

We present a series of cationic reagents that enable rapid cysteine arylation under mild conditions compatible with proteins and peptides. The highly polarized carbon-leaving group bond and attractive nucleophile-electrophile Coulombic interactions substantially accelerate the reaction, leading to unusually high-rate constants. The synthetic modularity of this approach allows for the direct coupling of structurally diverse functional motifs to cysteine residues of biomacromolecules with high efficiency. This simple, user-friendly chemistry enables fast bond formation between commonly used bioconjugation partners, thus greatly streamlining the workflow, and can be easily developed as convenient kits for chemical biology and medicinal chemistry applications.



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#57 - ENGINEERING AND CHARACTERIZING CHIMERIC DcuS/EnvZ HISTIDINE KINASE RECEPTORS AGAINST NOVEL LIGANDS

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Mr. Samuel Hinton, University of Oregon

Mr. Andrew Holston, University of Oregon

Mr. Philip Jimenez, University of Oregon

Dr. Calin Plesa, University of Oregon

Bacteria deploy a wide range of two-component systems comprised of a sensor histidine kinase (SHK) and cognate response regulator (RR) to interact with and respond to their environment. SHKs detect a wide scope of ligands and stimuli, both extra- and intracellularly, such as changes in osmolarity, temperature, pH, small metal ions, and chemical gradients of organic compounds. Generally, upon ligand binding, the SHK phosphorylates its respective RR, which influences gene expression in response. To date, approximately 3.5 million unique SHK proteins have been identified, but only a few hundred have been characterized with ligands identified. To bridge this gap, we take advantage of the high modularity of SHK sensory domain structure to engineer chimeric HKs. The sensory domain of the SHK, DcuS, is fused with the transmembrane and cytoplasmic domains of EnvZ. The chimera then communicates with an orthogonal RR, which mediates transcription of a synthetic gene circuit with superfolder green fluorescent protein (sfGFP) as the reporter. To find chimeras with novel functions we constructed a large library of mutagenized DcuS/EnvZ chimeras. Here, we screen both the original Dcus/EnvZ chimera and the mutagenesis library against the known ligands for endogenous DcuS as well as a panel of structurally similar chemicals. By measuring the log-fold change in fluorescence output relative to a no-ligand

control using flow cytometry, our study aims to identify new DcuS sensory domains with specificity for novel ligands. Our future studies aim to probe many different SHK chimeras with varying sensory domains against a large panel of ligands in order to characterize SHKs at scale.

#45 - FUNCTION-GUIDED PROXIMITY MAPPING IN C. ELEGANS DIRECTED BY LOCAL ELECTROPHILE RESPONSIVITY

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Dr. Amogh Kulkarni, Swiss Federal Institute of Technology Lausanne (EPFL)

Dr. Daniel A. Urul, Swiss Federal Institute of Technology Lausanne (EPFL)

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Leveraging promiscuous labeling enzymes, the current proximity-labeling methods are limited to head-counting protein residents in specific locales. Given that posttranslational modifications (PTMs) play crucial roles in cell signaling, we here established a method to identify electrophile-sensing functional proteins responsive to lipid-derived electrophiles (LDEs) in specific organs in live *C. elegans*. >70% of tissue-specific targets present novel responsivity independent of tissue-specific protein abundance. Gene knockdown by RNA interference in *C. elegans* indicated functional roles for responsive targets in animal survival, development, and stress management. One protein-isoform of interest (POI) was further studied because of its role we discovered in regulating the animal gut homeostasis under electrophile stress. Using target-specific labeling technology developed by our lab, this POI and its human ortholog were validated to have the conserved site(s)-specific LDE-sensing ability.

Subsequent biochemical and worm-based genetic investigations unmasked unexpected nuanced mechanisms whereby the organ-specific electrophile-sensing capability of this POI plays an indispensable role in global decision-making under stress.

#51 - AN ADVANCED CELL SIGNALING SYSTEM FOR DISCERNING CANCER INHIBITION MECHANISMS IN GANASPIS HOOKERII WASP VENOM

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Dr. Pooja Kr, Illinois State University

Dr. Nathan T. Mortimer, Oregon State University, Illinois State University

While venom use comprises a sizeable percentage of species on Earth, the complexity and variation of their components and the necessary volume required makes venoms an understudied and untapped resource for potential drug candidates. The parasitic wasps of *Drosophila melanogaster* are one such understudied group. These wasps seek out immature *Drosophila* larvae to use as hosts for their progeny, injecting an egg, and venom, which previous studies have shown protects the wasp egg from the larval immune system. As very little is known about the activities of these wasp venoms, we wanted to test the effects of venom on pathological processes such as tumorigenesis. Independent prior work has established a *Drosophila* tumor model using RasV12 expression and Scribble loss in conjunction with the MARCM (Mosaic Analysis with a Repressible Cell Marker) technique. RasV12 is an oncogenic gain of function Ras mutant that drives hyperplastic tumor formation. Scribble (*scrib*) is essential for establishing and regulating apicobasal polarity and, when lost, causes small benign tumors. MARCM allows for the generation of a mitotically homozygous cell with the capability to drive gene expression. When RasV12 expression and homozygous *scrib* loss are concurrently induced with the MARCM technique, larvae form aggressive invasive

tumors that share many characteristics with human cancer. However, when these larvae are infected with venom from *Ganaspis hookerii* wasps, the tumors are significantly reduced. This suggests that the venom is either acting through an apoptotic signal or as a cell cycle inhibitor. In an effort to understand the mechanism by which the venom is influencing the cancerous phenotype, we have devised a genotypic signaling system to introduce apoptosis and cell cycle progression reporters into our tumor model. We then infect the larvae with *G. hookerii* and observe reporter activity. The first reporter line uses GC3Ai, which is a GFP based apoptosis sensor that reports caspases via a modified GFP molecule with a strategically placed caspase cleavage site. If there are caspases, the GFP becomes active, and if there are no caspases, the GFP remains inactive. The other reporter line utilizes the fly fluorescent ubiquitination-based cell cycle indicator (FUCCI) system. FUCCI uses fluorescent protein components that are tagged with degrons for ubiquitin ligases present only during specific cell cycle phases, to signal what phase the cells are currently in. Specifically, EGFP is tagged with an E2F1 fragment containing the target degron for the S-phase dependent ubiquitin ligase CRL4Cdt2, which means the EGFP is expressed in cells in G2, M, and G1 phase. In addition, mRFP1 is tagged with a CycB fragment containing the target degron for the ubiquitin ligase APC/C, which is active from mid-mitosis through G1 and marks cells in S, G2, and early M phase. So cells in S phase will be red, G2 and M will be yellow, and late M and G1 will be green. If the venom stops the cell cycle, this enables us to discern when the cell cycle arrest occurred. These methods will allow us to better understand the mechanisms by which the wasp venom inhibits tumorigenesis, and could provide insight on potential new cancer treatments.

#73 - NOVEL ANTI-CB1 ANTIBODIES AND THEIR USE IN STUDYING CB1 GLYCOSYLATION AND SUBCELLULAR LOCALIZATION

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The cannabinoid type 1 receptor (CB1) is a neuroreceptor that is highly expressed throughout the central nervous system and involved in synaptic plasticity. Despite its important role in normal brain physiology, aspects of this receptor remain difficult to study, in part due to a lack of effective tools and several disordered regions within the receptor. To address this, new anti-CB1 monoclonal antibodies were generated, characterized, and validated to assist in the study of CB1 structure and function. We find one of these antibodies is unique - it only binds to an artificially truncated version of CB1, where it binds with high affinity and specificity. Moreover, the epitope to which it binds is short (4 amino acid) and must be located N-terminally. We find this antibody/epitope combination can be used for several applications, including western blot, immunoprecipitation, and immunofluorescence. We are currently exploring its use as a “tag” to assess the impact of glycosylation on CB1 receptor expression at the plasma membrane.

#69 - STEADY STATE TRANSMEMBRANE WATER EXCHANGE IN *S. CEREVISIAE* WITH TWO SITE EXCHANGE KINETICS

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Johnathan Dutra, Portland State University

Joe Armstrong, University of Washington

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The rate constant for steady state water efflux, k_{io} , can be measured using contrast enhanced magnetic resonance (MR). k_{io} is known to correlate with the cellular energy molecule ATP making it a probable biomarker for metabolic diseases such as cancer. Measuring k_{io} in tumors illuminated regions of heterogony potentially allowing for targeted treatment. This research is focused on understanding the molecular metabolic origins of differences in k_{io} . Using yeast (*S. cerevisiae*) as a model organism grown in a chemostat we can obtain samples of cells in a uniform metabolic state. This provides an excellent platform for investigating the factors that govern k_{io} . Yeast cells were grown under respiring and fermenting metabolic regimes in chemostat bioreactors. The effect of mitochondrial activity was investigated in yeast though the use of metabolic modulators. The results suggest that mitochondrial activity may significantly influence k_{io} .

#27 - DIFFERENTIAL DYNAMICS EXPLOITABLE FOR SELECTIVITY BETWEEN HOMOLOGOUS RNA-BINDING PROTEINS WITH COVALENT LIGANDS

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Julia Haas, University of Notre Dame

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One in five disease-annotated mutations occur in RNA-binding proteins (RBPs); yet this class of proteins is widely considered “unligandable” and “undruggable”, as traditional non-covalent small molecule screens often yield non-selective ligands. This is attributed to several features of RBPs, such as: dynamic and disordered structures, lack of traditional ligand binding pockets, and high structural conservation. One way to overcome these challenges is to utilize

covalent small molecule ligands. Covalent ligands have several advantages, such as site-directed binding, increased potency due to covalent bond formation, and the ability to target transient binding pockets.

As proof-of-concept, my project focuses on discovering small molecules for an RBP model system, heterogenous nuclear ribonucleoproteins (hnRNPs) H and F. The model system was selected for its highly characterized tandem quasi-RNA Recognition Motifs (qRRMs 1,2). The qRRMs1,2 of hnRNP H and F have identical tertiary structures and greater than 90% sequence similarity. The difference between the protein homologs is a single proline to alanine mutation in the inter-domain linker, which leads to differential dynamics in the two proteins. *I propose that hnRNPs H and F can be selectively targeted by exploiting their differential dynamics using covalent ligands.*

To test this hypothesis, I identified covalent small molecules in the literature that targeted three conserved cysteine residues in qRRMs1,2 of hnRNP H and F in cell lysates. I then screened the ligands *in vitro* against qRRMs1,2 of hnRNP H and F, utilizing intact protein mass spectroscopy. In contrast to the cell lysate screens, all the covalent ligands formed covalent bonds at higher rates to hnRNP H than hnRNP F. One covalent ligand, fragment 12 (F12), had two-fold selectivity for hnRNP H over F and for cysteine 22 (C22) over the other two cysteines. By commercial and synthetic SAR, we have extensively explored the covalent warhead and the molecular recognition motif to identify the critical components for ligand selectivity. Furthermore, diversification of F12 has led to a suite of derivatives with increased selectivity.

We propose that slower protein motions, as those observed in hnRNP H, lead to more pocket-like structures and thus, higher rates of covalent bond formation. Our future work will test this hypothesis using protein constructs with mutations known to affect the dynamics, which

will be screened against our suite of derivatives from initial SAR. Taken together, our results indicate that RBPs are indeed targetable with covalent ligands and that selectivity can be achieved for even highly homologous RBPs.

#9 - TARGETED DEGRADATION OF APOPTOSIS SIGNAL-REGULATING KINASE 1 USING SELONSERTIB-BASED PROTACs: A PROMISING APPROACH FOR THE TREATMENT OF NAFLD/NASH

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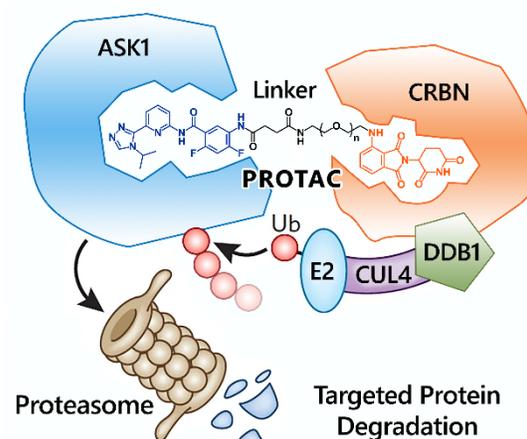
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Apoptosis signal-regulating kinase 1 (ASK1), a mitogen-activated protein kinase kinase kinase (MAP3K) in the c-Jun N-terminal kinase pathways is essential to elevate hepatic inflammation and fibrosis.¹ Inhibition of ASK1 attenuates hepatocyte inflammation, fibrogenesis, cell death and improves NAFLD/NASH.² Very recently, a small molecule drug selonsertib (GS-4997, Gilead Sciences) showed promising anti-fibrotic activity by



inhibiting the downregulation of ASK1/MAPK pathway, but failed in Phase III clinical trial of fibrosis improvement in NASH patients.³ Henceforth, the development of an alternative approach to treat NAFLD/NASH is of utmost importance.

Targeted protein degradation (TPD) by PROteolysis TArgeting Chimeras (PROTACs) has emerged as an exciting area in both basic biological discovery and drug development.⁴ PROTACs are heterobifunctional molecules made up of a target protein-binding ligand and an E3-ligase recruiting ligand connected by a suitable intervening linker.⁵ Because of their unique mode of action, PROTACs have a number of advantages over small-molecule inhibitors, including catalytic activity and the ability to address non-enzymatic functions of proteins.⁶

Herein, we designed and synthesized a set of hetero-bifunctional PROTAC molecules for the targeted proteasomal degradation of ASK1 by hijacking E3-ubiquitin ligases cereblon and VHL.⁷ Among several synthesized compounds, dASK1 showed higher degradation efficiency within 1-100 nM, leading to the fast, efficient, and prolonged degradation of ASK1 in both HepG2 and HEK293A cell lines. The *in vitro* results have been carefully validated using molecular docking and the binding free energy of the ternary complex was estimated by MM-GBSA calculation. We are also validating our primary results in *in vivo* disease model. Thus, the targeted degradation of ASK1 has great potential and may ultimately be used to achieve lasting, improved human health.

Controlling small molecule probe's activity with higher precision has been the Holy Grail of chemical biology research.⁸ Light with high spatiotemporal resolution has been widely used in chemical biology, neurobiology and disease treatment.⁹⁻¹⁰ We are stepping forward to develop light-controllable PROTACs to achieve spatiotemporal control over targeted protein degradation. Because light can function at

millisecond and sub-micrometer resolutions, we envision that this strategy will show great promise in the development of next-generation photopharmacological tool to treat localized diseases.

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#71 - VISUALIZATION OF RNA-PROTEIN INTERACTIONS VIA CONFOCAL RAMAN SPECTROSCOPY

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RNA-protein interactions are essential for cell processes ranging from RNA localization to proper protein function. While several methods have been developed to analyze binding between RNA and proteins, most of these methods lack structural information about how these interactions take place. To better understand these interactions, we aim to develop a confocal Raman spectroscopy method to characterize these binding interactions *in vitro*. This method compared to standard Raman spectroscopy can allow for the detection of low concentrations of RNA using either thiol functionalized RNA or a DNA-RNA hybridization approach to immobilize the RNA within silica beads that greatly enhance the Raman signal. We will present progress toward the silica bead derivatization chemistry and the study of a

model RNA-protein interaction between the thrombin protein and an RNA aptamer.

#87 - RAF INHIBITORS EXHIBIT EXPLOITABLE COMPLEX-SPECIFIC SELECTIVITY

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Small molecule kinase inhibitors are often classified according to specific conformations that kinases adopt upon inhibitor binding. These changes in conformation can lead to activity-independent effects by perturbing inter- and intra-molecular interactions of kinase regulatory domains. Conformation-selective small molecule inhibition of Raf kinases, serine/threonine kinases that are a key component of the Ras-Raf-MEK-ERK MAPK pathway, lead to profound changes in Raf protein:protein interactions. Active Raf kinases are associated with a protein complex consisting of a 14-3-3 stabilized Raf dimer that interacts with Ras, a small GTPase that acts as a Raf activating "switch". Inactive Raf exists as a monomer that is also stabilized by a distinct 14-3-3 dimer and an autoinhibitory interaction with Raf's substrate, Mek. It is known that Raf inhibitors promote dimeric, active-like, Raf complexes resulting in paradoxical activation of the pathway at substoichiometric concentrations of Raf inhibitor. We quantified Raf inhibitor potency across KRas mutant/Raf wild type cancer cell lines and observed a broad range of inhibitor potency with IC50s of downstream signaling from low nanomolar to micromolar. We observed that intracellular Raf activity, as measured by phosphorylation of its substrate Mek, correlates with intracellular Raf inhibitor potency such that the more active Raf is, the easier it is to inhibit. This correlation persists upon A115-promoted Ras activation within cells expressing WT Ras and the protein

construct, CIAR (Chemically Inducible Activator of Ras). We found the selectivity of Raf inhibitors for activated forms of Raf seem to be driven in large part by selectivity for specific Raf complexes and phosphoforms. We found that complexes associated with inactive forms of Raf are insensitive to inhibition while complexes associated with active forms are inhibitor-sensitive. Altering the landscape of Raf complexes within cells can be achieved using allosteric Mek inhibitors. It is known that some Mek inhibitors promote inactive Raf:Mek complexes while others promote active Raf complexes. We observed that perturbing Raf:Mek complexes using Mek inhibitors also altered the sensitivity of Raf inhibitors toward inhibition of the same complexes resulting in both synergistic and antagonistic relationships between Raf and Mek inhibitors. We demonstrate that the mechanisms behind the complex-specificity of Raf inhibitors can be leveraged to inform synergistic partners.

#39 - CELL SPECIFIC PHOTOPHARMACOLOGY IN HUMAN ISLETS USING OPTICALLY-CLEAVABLE TARGETED (OCT)-LIGANDS

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 Alexander E.G. Viray, Oregon Health & Science University
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N-acylethanolamides (NAEs) are a lipid family that target cannabinoid receptors and are known mediators of glucose-stimulated insulin secretion (GSIS) from beta-cells in pancreatic islets. Due to the diversity observed in NAE structure and pharmacology, coupled with the expression of multiple NAE-sensitive cannabinoid receptors in beta-cells, our understanding of NAE signaling is limited by our inability to control their actions in time and space. To disentangle how NAE signaling regulates beta-cell excitability, we developed the Optically-Cleavable Targeted (OCT)-ligand approach, which combines the spatial resolution of self-labeling protein tags

(SNAP-tags and Halo-Tags) with the temporal control of photocaged ligands. By linking a photocaged NAE to an o-benzylguanine motif, NAE signaling can be directed towards genetically-defined SNAP-tag expression. We designed a probe to release palmitoylethanolamide (PEA), a GPR55 agonist known to stimulate GSIS. When targeted to the plasma membrane of INS-1 cells, OCT-PEA revealed that GPR55 stimulates beta-cell Ca^{2+} activity via phospholipase C. In order to use our platform in intact islets, we developed an AAV to selectively express either SNAP-tags or Halo-tags on the surface of beta-cells while suppressing alpha-cell expression. Using an improved second-generation OCT-PEA, we effectively labeled Halo-tags on the beta-cell surface in intact islets and stimulated Ca^{2+} oscillations at 11 mM glucose. Moving forward, the OCT-ligand approach can be expanded to other ligands and receptors, and will open up new experimental possibilities in targeted pharmacology.

#81 - BREAKING BAD: A VERSATILE STRATEGY FOR PRODUCTION OF PERMANENTLY PHOSPHORYLATE INTRINSICALLY DISORDERED PEPTIDES OR PROTEINS

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Over 30% of proteins in the higher eukaryotic proteome contain intrinsically disordered proteins (IDPs), characterized by their structural flexibility and lack of stable 3D structures. They have essential roles in cellular signaling and regulation, mediating interactions with hub proteins and serving as regulatory switches through reversible phosphorylation, influencing conformational dynamics, function, and allosteric interactions. However, producing phosphorylated IDPs faces two substantial challenges. First, IDPs themselves are often proteolyzed, unstable and aggregation prone,

and sometimes toxic to the expression hosts. Second, there is the challenge of installing phosphoserine into IDPs at the targeted location and preventing it from being hydrolyzed during expression and purification. Here, we describe development of an innovative expression strategy that overcomes these challenges. We showcase the utility of this strategy by expressing phosphorylated BAD (Bcl2-associated agonist of cell death), a proapoptotic IDP that in its unmodified form primes cells for apoptosis, but when phosphorylated at up to 3 sites is inactivated by sequestration with 14-3-3. It is also notorious for its instability when over-expressed in recombinant hosts and so for two decades it has eluded *in vitro* characterizations. To express BAD phosphorylated at Ser136 in *E. coli*, it was fused with a carrier protein to direct its expression into inclusion bodies to prevent degradation from cellular proteases. We then coupled this expression of BAD with genetic code expansion (GCE) systems to direct the site-specific translational installation of non-hydrolyzable phosphoserine (nhpSer) at site 136 using PermaPhos, an efficient GCE platform where *E. coli* cells biosynthesize the nhpSer amino acid and encode it into proteins system. We chose to install nhpSer at site 136, instead of native phosphoserine, to prevent hydrolysis of phosphorylated BAD during expression and purification. After purification and proteolytic cleavage of BAD-nhpSer136 from the inclusion body tag, several milligrams per liter culture was obtained. This methodology can be easily adapted for expression of many other phosphorylated IDPs ranging from few to hundreds of amino acids, making them accessible for downstream *in vitro* characterization. Further, because the proteins are phosphorylated with a stable, functional mimic of pSer, transfection into mammalian cells to evaluate *in vivo* function of specific phosphorylated forms of IDPs are possible.

#91 - A CHEMICAL BIOLOGY APPROACH TO STUDYING THE PROTEOME OF HUMAN MACROPHAGES

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 Dr. Balyn Zaro, University of California San Francisco

Macrophages play a vital role in the innate immune system, identifying and destroying unwanted cells. They do so through phagocytosis, a process which allows macrophages to eat and digest other cells. Phagocytosis is highly regulated, partly by interactions between proteins on the cell surface of macrophages and their target cells. Research shows that some macrophage surface proteins are receptors which transmit a downstream signal upon interaction with a corresponding ligand protein. These ligands are expressed by all cells in the body and can result in the transmission of an inhibitory 'Don't eat me' (DEM) signal within the macrophage. Healthy cells express DEM ligands to prevent their premature clearance from the body. As these cells age or acquire mutations, they lose expression of DEM ligands, leading to macrophage-mediated removal. However, it has been shown that cancer cells instead upregulate expression of DEM ligands, preventing their phagocytosis. Therapeutics against DEM ligands are effective when a patient responds, but a large percentage do not, highlighting a gap in knowledge about other factors contributing to regulation of macrophage phagocytosis. During my thesis work, I developed a protocol to culture primary human macrophages isolated from patient blood and feed them human cancer cells. My protocol then allows for separation of macrophages which successfully phagocytose (Eaters) from those which do not (Noneaters). As demonstrated above, proteins play a critical role in the regulation of macrophage phagocytosis. Therefore, I use mass spectrometry to compare the proteome of Eaters and Noneaters, looking for proteins which show differential abundance. Interestingly, one of the most differentially abundant proteins in the Eaters was EPCAM,

which mediates cell to cell adhesion in epithelia. Macrophages unexposed to cancer cells and Noneaters did not have any EPCAM present, consistent with literature. This result suggests Eaters may acquire the protein from their cancer cell meal, SW620s, which are a colorectal cancer known for high EPCAM expression. I then used flow cytometry to determine that EPCAM is not just within the Eaters but is present on their cell surface. The assay returned a gradient in signal, with Noneaters showing no labeling, Eaters showing robust signal, and an intermediary population (Nibblers) falling between. I am currently following up on this finding via microscopy and RT-PCR to determine if Eaters and Nibblers are acquiring EPCAM via trogocytosis, sampling of the SW620 surface, or expression from acquired virulent genetic material from the cancer cells. In addition, I am following up on other differentially expressed targets and repeating the assay with added enrichment of surface proteins to increase their resolution. Through this work, I aim to elucidate what occurs when a macrophage undergoes phagocytosis and how it can be levered to identify new therapeutic targets.

#33 - CONTROL CELL SIGNALING PATHWAYS USING DESIGNED DE NOVO PROTEIN BINDERS

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 Mr. David Lee, University of Washington
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 Prof. David Baker, University of Washington
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 Prof. Beth L Roman, University of Pittsburgh
 Prof. Eunhee Choi, Columbia University
 Prof. Xiaochen Bai, UT Southwestern Medical Center

The continuous advancements in structural determination techniques have significantly increased the availability of high-resolution

protein structures each year. The integration of AI-enabled structure prediction has further expanded the scope of target domains, while breakthroughs in de novo protein design have demonstrated the capacity to craft selective binders targeting specific sites. This collective progress has opened new avenues for the development of protein modulators capable of precisely regulating desired signaling pathways. Here, I will report our progress in modulating signaling pathways through controlling receptor protein conformation and oligomerization states.

#95 - IDENTIFICATION OF LAMIN PROTEIN-PROTEIN INTERACTORS BY SMALL MOLECULE MEDIATED PROXIMITY LABELLING

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The network of intermediate filament proteins that forms the nuclear lamina imparts rigidity and structural integrity to the mammalian cell nucleus. Nuclear lamins are the major component proteins at the nuclear lamina and participate in essential nuclear functions that include mechanosensing, DNA repair, chromatin regulation, gene transcription and stem cell regulation. Dysfunction of the lamina leading to disruption of its protein-protein interactions (PPI) has been implicated in various pathological processes. Existing approaches to study the PPIs of the nuclear lamina are not appropriate for detection of native PPIs in living cells as they rely on unsuitable biochemical methods or artifact-prone overexpression systems. We hypothesize that small molecule probes will permit the identification of lamin-protein interactions with minimal disturbance to its endogenous function.

We previously identified a novel small molecule LBL1 that specifically target the nuclear lamina. Based on LBL1, we developed a novel clickable photoaffinity probe LBL1-PCF to label nuclear lamina in live cells. The azide group in LBL1-PCF further allows a strain-promoted azide-alkyne cycloaddition (SPAAC) with a secondary trifunctional photoaffinity probe in live cells. By employing this dual-probe system, we captured and identified a number of novel lamin interactors by mass spectrometry. These novel interactors were validated through a series of independent orthogonal assays. Our results provide novel mechanistic insights into the disease etiologies of laminopathies.

#55 - IDENTIFICATION OF THE MOTIF(S), MECHANISM(S), AND PATHWAYS(S) RESPONSIBLE FOR D1 DOPAMINE RECEPTOR RECYCLING

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Dopamine is a neurotransmitter that regulates many aspects of human physiology including movement, memory, and reward. Dysregulation of dopamine can lead to neurodegenerative diseases like schizophrenia, Parkinson's, and addiction. Dopamine exerts its effects by activating dopamine receptors, members of the G protein-coupled receptor (GPCR) family of signaling proteins. Cellular trafficking of dopamine receptors is a critical facet of their regulation. When brought into the cell, dopamine receptors are either trafficked to the lysosome or recycled back to the cell membrane. The D1 dopamine receptor (D1DR) is known to recycle back to the cell membrane; however, the motif(s), mechanism(s), and pathways(s) responsible for this ability have not been identified. This project utilizes a novel chimeric approach to uncover the specific amino acid(s), complex(es), and pathway(s) that make this recycling ability possible. A better understanding of D1DR recycling could allow for a better

understanding and treatment of neurodegenerative diseases and help to uncover the relationship between receptor recycling and tolerance.

#67 - VENOMS AND THEIR TOLL (LIKE RECEPTORS)

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Our lab is focused on understanding cell signaling in the context of host-parasite interactions. We use the *Drosophila*-parasitoid wasp system, in which endoparasitoid wasps infect *Drosophila* larvae and, during infection, transfer both an egg and venom into the host. Endoparasitoid wasps strategically utilize specific proteins within their venom to ensure the successful development of their offspring in the host environment. Elucidating the contents of this venom will allow us to understand the mechanisms by which this obligate parasitoid has evolved to be able to defend its offspring against the host, particularly the signaling events of the host immune response, mounted in an act of self-preservation to eliminate the parasitoid. While a few venom mechanisms have been previously described, we hypothesize that the parasitoid *Ganaspis hookeri* (strain G1) takes a novel approach to circumvent this response. We have found that G1 venom contains multiple protein mimics of Toll-like receptor (TLR) proteins. TLRs are implicated in numerous innate immune signaling pathways, including the anti-parasitoid response, and are widely conserved. Our predicted structural alignments suggest that G1 makes use of biochemical mimicry to circumvent TLR signaling, which is vital to this host-parasitoid interaction. We present comparisons of a set of disparate, AlphaFold generated, G1 venom protein predictions to a solved crystal structure of the ectodomain of Fly Toll bound to its endogenous cytokine, Spätzle. This analysis will allow us to make predictions for

future structure-function testing in cell culture and *Drosophila* larvae.

#77 - SMALL MOLECULE INHIBITORS OF PROTEIN KINASE C THETA (PKC θ) SIGNALING PROMOTE PROCOAGULANT PLATELET GENERATION: INSIGHTS FROM PHOSPHOPROTEOMICS AND SYSTEMS ANALYSIS

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Background: Platelet activation is pivotal in both physiological hemostasis and thrombosis, where multiple mechanisms regulating platelet activity are imperative. Protein Kinase C (PKC) isoforms are key regulators in platelet activation, contributing to hemostasis, inflammation, and thrombosis. While specific PKC family members have well-defined roles in platelet function, the roles of PKC θ (PRKCQ, PKC theta) remain less defined.

Objectives: To determine mechanisms of PKC θ signaling that orchestrate procoagulant platelet generation.

Methods: Platelets were prepared from healthy human donors for *ex vivo* biochemical, physiological, and systems biology studies. Platelet signaling and platelet function were

studied following stimulation with GPVI agonist crosslinked collagen-related peptide (CRP-XL). To investigate PKC θ inhibition mechanisms in platelets, small molecule inhibitors targeting the PKC θ isoform (CC-90005 and C20) were employed. This was accompanied by a range of functional assays including platelet aggregation, static adhesion, live adhesion, cytosolic Ca²⁺ measurement, and fibrin generation to evaluate platelet functionality. Additionally, Western blot analysis using antisera specific to phosphorylation sites on PKC θ isoforms and PKC substrates.

Results: Phosphoproteomics analyses of agonist-stimulated platelets quantified significant, site-specific changes in the phosphorylation of PKC isoforms as well as numerous PKC substrates. In addition to well-established phosphorylation events (e.g., PKC δ Y311), we noted prominent phosphorylation on the C-terminus PKC θ at Ser685. After generating and characterizing antisera specifically reactive to PKC θ phospho-Ser685, Western blot analysis determined that PKC θ Ser685 phosphorylation occurred later following GPVI platelet activation (>5 min). Pretreatment of platelets with inhibitors specific PKC isoforms revealed that PKC α/β , PKC θ , and, to a lesser extent, PKC δ activities determine PKC θ Ser685 phosphorylation. Parallel platelet function analyses found that inhibition of PKC θ had only minor effects on platelet spreading and aggregation. However, PKC θ inhibition in the presence of calcium ions upregulated platelet phosphatidylserine (PS) exposure, as measured by flow cytometry with FITC-lactadherin. This was accompanied by an increase in platelet procoagulant activity, where PKC θ inhibition reduced the lag time for fibrin formation. Furthermore, using the low-affinity Ca²⁺ indicator Fluo-5N-AM, which binds only very high concentrations of Ca²⁺, PKC θ inhibition led to an increase in intracellular Ca²⁺ concentration. Fluorescence microscopy also found that PKC θ inhibition significantly augmented the proportion of procoagulant platelets and microparticles

under these conditions. Additionally, compared to the control group, the use of cyclosporin A (CsA) reduced the production of procoagulant platelets, but the addition of CC-90005 and C20 rescued this phenomenon, with signal intensity surpassing that of the control group.

Conclusions: In summary, our study proposes a model wherein PKC δ and PKC α/β activities induce the phosphorylation of PKC θ at S685, influencing mitochondrial calcium signaling and subsequently leading to an increased intracellular calcium ion concentration, which, in turn, supports procoagulant platelet generation. This observation underscores the need for vigilant monitoring of thrombotic events when contemplating the therapeutic application of PKC θ -related inhibitors in patients with pertinent immune disorders in the future.