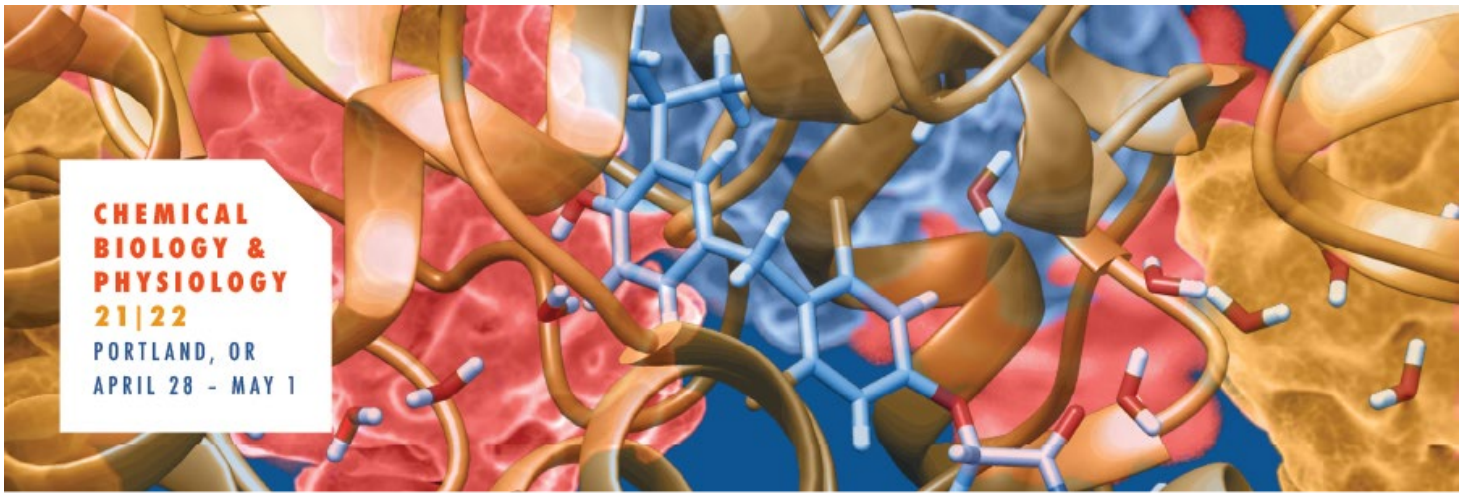
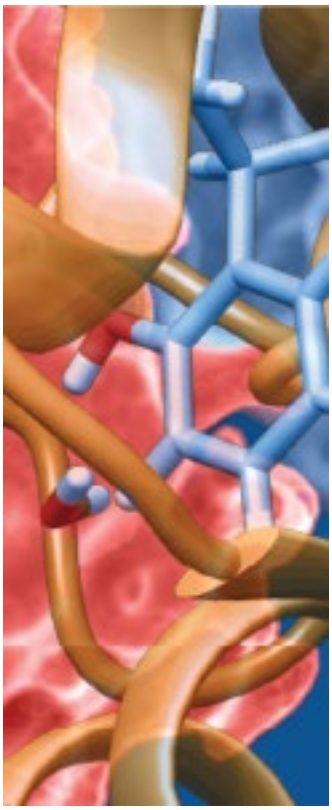


Program and Abstracts

April 28 – May 1, 2022
Oregon Health & Science University
Portland, OR USA

OHSU



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SPECIAL THANKS TO:
Nick Crampton and Ken Welcome for AV support

All activities take place at the:
1st Floor of the Knight Cancer Research Building
Oregon Health & Science University
2720 S.W. Moody Avenue, Portland, Oregon 97201 USA

THURSDAY – APRIL 28

11:00 – 13:00	Registration and Welcome
13:00 – 13:10	Opening Remarks

SESSION 1: BIOINORGANIC CHEMISTRY
Chair: Vickie DeRose

13:10 – 13:45	**Hosea Nelson**, California Institute of Technology, Pasadena *The Challenge of Making Every Vaccine Safer and More Effective: Modulating Innate Immunity Via Signal Processing*
13:45 – 14:00	Short Talk: **Ananya Rakshit**, University of Colorado, Boulder *Zinc signaling and metal ion homeostasis in mammalian cell cycle*
14:00 – 14:35	**Kathy Franz**, Duke University *Infectiously Inorganic: A Metallocentric View of Antimicrobial Activity*
14:35 – 14:50	Short Talk: **Kathrine Rush**, Oregon Health & Science University *Se EXAFS reveals direct substrate binding function for auxiliary iron-sulfur clusters in radical-SAM catalyzed thioether crosslinking*
14:50 – 15:10	Break
15:10 – 15:25	Short Talk: **Eva Ge**, University of California, Berkeley *New therapeutic platforms for targeting metal nutrient homeostasis vulnerabilities in cancer*
15:25 – 16:00	**Elisa Tomat**, University of Arizona *Bioconjugate Strategies to Target the Iron Core of Cancer*
16:00 – 16:15	Short Talk: **Hannah Pigg**, University of Oregon *Variations in Nucleolar Stress Produced by Small-Molecule Pt(II) Compounds*
16:15 – 16:55	Flash Talks: Poster presenters
16:55 – 17:15	Break

KEYNOTE SPEAKER
Chair: R. David Britt

17:15 – 18:15	**Alison Butler**, University of California, Santa Barbara *Origins and significance of chirality in siderophores: From marine microbes to pathogens*

18:15 – 20:00	Reception

FRIDAY – APRIL 29

SESSION 2: MOLECULAR SWITCHES
Chair: Carsten Schultz

08:45 – 09:20	**Alexander Deiters**, University of Pittsburgh *Molecular Switches for Protein and Nucleic Acid Function*
09:20 – 09:55	**Ming Chen Hammond**, University of Utah *Riboswitching on the light: fluorescent biosensors for molecular imaging and discovery*
09:55 – 10:10	Short Talk: **Jason Zhang**, University of Washington *De novo designed tools to map the activity/function of endogenous and oncogenic Ras signaling*

10:10 – 10:30	Break
10:30 – 11:05	**Takanari Inoue**, Johns Hopkins University *Total synthesis of innate immune functions in inert and artificial cells*
11:05 – 11:20	Short Talk: **Aurélien Laguerre**, Oregon Health & Science University *Regulation of Calcium Oscillations in β-Cells by Co-activated Cannabinoid Receptors*
11:20 – 12:00	Flash Talks: Poster presenters
12:00 – 13:00	Lunch Meet the Speakers
13:00 – 14:30	In-Person Poster Session I – sponsored by Thermo Fisher Scientific

SESSION 3: IMAGING AND BIOSENSORS
Chair: Kimberly Beatty

14:30 – 15:05	**Loren Looger**, HHMI, University of California - San Diego *New Tools for Monitoring & Manipulating Cellular Activity*
15:05 – 15:20	Short Talk: **Brittany White-Mathieu**, Cornell University *Lipid Expansion Microscopy*
15:20 – 15:55	**Kim Bonger**, Radboud University, The Netherlands *Chemoenzymatic tools for targeted drug delivery*
15:55 – 16:15	Break
16:15 – 16:30	Short Talk: **Pratik Kumar**, HHMI Janelia Research Campus *Multifunctional fluorophores as molecular tools beyond imaging*
16:30 – 17:05	**Bryan Dickinson**, University of Chicago *Continuous directed evolution of molecular interactions: fundamental studies and applications*
17:05 – 17:15	Break

KEYNOTE SPEAKER
Chair: Janelle Tobias

17:15 – 18:15	**Hiro Suga**, University of Tokyo, Japan *Display of pseud-natural peptides and products*

SATURDAY – APRIL 30

SESSION 4: IMMUNE RESPONSES
Chair: Mike Cohen

08:45 – 09:00	Short Talk: **Scotty Farley**, Oregon Health & Science University *Exploring the roles of host lipids during SARS-CoV-2 infection*
09:00 – 09:35	**Aaron Esser-Kahn**, University of Chicago *The Challenge of Making Every Vaccine Safer and More Effective: Modulating Innate Immunity Via Signal Processing*
09:35 – 10:10	**Lingyin Li**, Stanford University *Chemical biology of the innate immune STING pathway*
10:10 – 10:30	Break
10:30 – 10:45	Short Talk: **Balyn Zaro**, University of California, San Francisco *A Chemical Biology Approach to Studying Innate Immune Signaling*
10:45 – 11:20	**Dan Bachovchin**, Sloan Kettering Institute *Selective Activators of the CARD8 Inflammasome*
11:20 – 12:00	Flash Talks: Poster presenters
12:00 – 13:00	Lunch Meet the Editors
13:00 – 14:30	In-Person Poster Session II Virtual Poster Session - sponsored by Thermo Fisher Scientific

SESSION 5: PROTEIN CHEMISTRY
Chair: Ryan Mehl

14:30 – 15:05	**Christina Woo**, Harvard University (remote) *Discovery of a degron for the thalidomide binding domain of cereblon*
15:05 – 15:20	Short Talk: **Robert Dorn**, University of California, Irvine *Chemically activated proximity labeling with bioorthogonal phosphines*
15:20 – 15:55	**Keriann Backus**, University of California, Los Angeles (remote) *Expanding the activity-based chemoproteomic toolbox*
15:55 – 16:15	Break
16:15 – 16:30	Short Talk: **Shizhong Dai**, University of California, San Francisco *New pharmacological strategies for tuning G protein signaling*
16:30 – 17:05	**Eranthie Weerapana**, Boston College *Chemical-proteomic strategies to investigate reactive cysteines*
17:05 – 17:20	Short Talk: **Rachel Franklin**, Oregon State University *Developing Top-down Mass Spectrometry Methods for the Investigation of Genetically Engineered Protein Systems*
17:20 – 17:30	Break

KEYNOTE SPEAKER
Chair: Annie Gilbert

17:30 – 18:30	**Barbara Imperiali**, Massachusetts Institute of Technology *The 'ins and outs' of initiating glycoconjugate biosynthesis at the membrane frontier*

SUNDAY – MAY 1

SESSION 6: CHEMICAL PHYSIOLOGY
Chair: Mike Cohen

08:45 – 09:20	**Arvin Dar**, Icahn School of Medicine at Mount Sinai *Targeting RAS-Dependent Cancers with Chemical Switches and Molecular Glues*
09:20 – 09:35	Short Talk: **Grace Wang**, Caltech *Bio-orthogonal Noncanonical Amino acid Tagging Reveals Novel interspecies Antagonism*
09:35 – 10:10	**Vanessa Franke-Ruta**, HHMI/The Rockefeller University *Making sense of scents: structural insights into odor detection*
10:10 – 10:30	Break
10:30 – 11:05	**Scott Sternson**, Janelia, HHMI, University of California, San Diego *Chemogenetics for research and potential translational applications*
11:05 – 11:20	Short Talk: **Tongil Ko**, New York University *Photoactivated proteolysis targeting chimeras (PHOTACs) for light-activated degradation of synaptic protein CAMKII in hippocampal tissue*
11:20 – 11:55	**Ratmir Derda**, University of Alberta, Canada *Genetically-Encoded Chemistry in Chemical Biology*
11:55 – 12:05	Break

KEYNOTE SPEAKER
Chair: Alex Eddins

12:05 – 13:05	**Tobias Meyer**, Weill-Cornell Medical College *Coordination of cortical actin and ER-PM contact sites directs receptor signaling to the front during cell migration*

13:05 – 13:15	Closing Remarks
13:15	Lunch

SELECTIVE ACTIVATORS OF THE CARD8 IFLAMMASOME

Dan Bachovchin –Department of Chemical Biology, Sloan Kettering Institute, USA
bachovcd@mskcc.org

Inflammasomes are multiprotein complexes that sense intracellular danger signals and induce pyroptosis. CARD8 and NLRP1 are related inflammasomes that are repressed by the enzymatic activities and the protein structures of the dipeptidyl peptidases 8 and 9 (DPP8/9). Potent DPP8/9 inhibitors such as Val-boroPro VbP) activate both NLRP1 and CARD8, but chemical probes that selectively activate only one have not been identified. Here, we report a small molecule called CQ31 that selectively activates CARD8. CQ31 inhibits the M24B aminopeptidases prolidase (PEPD) and Xaa-Pro aminopeptidase 1 (XPNPEP1), leading to the accumulation of proline-containing peptides that inhibit DPP8/9 and thereby activate CARD8. NLRP1 is distinct from CARD8 in that it directly contacts DPP8/9's active site; these proline-containing peptides, unlike VbP, do not disrupt this repressive interaction and thus do not activate NLRP1. We expect that CQ31 will now become a valuable tool to study CARD8 biology

EXPANDING THE ACTIVITY-BASED CHEMOPROTEOMIC TOOLBOX

Keriann Backus –Department of Chemistry and Biochemistry, University of California Los Angeles, USA
kbackus@mednet.ucla.edu

Chemical probes are powerful tools for characterizing protein function, which offer the advantages of producing graded effects (both agonism and antagonism) and acute application, features that are well suited to the study of essential genes and post translational processes. Despite their proven utility, most human proteins (>90%) lack selective chemical probes, and entire classes of proteins remain 'undruggable.' Prior chemoproteomics studies have demonstrated that the human proteome contains hundreds and quite possibly even thousands of small molecule-targetable, termed ligandable, cysteine residues. However, what remains unclear is whether and how the attached probes alter protein function. Combining proteomics, genomics, and covalent probes, our research aims to decipher the functions of these numerous ligandable cysteines, and by doing so we will generate a roadmap for the use of covalent probes to study and manipulate protein function. Here I will discuss the development of enhanced chemoproteomic methods that improve the throughput and our progress towards a proteogenomic platform to identify functional and druggable acquired cysteine residues as a new approach to develop precision therapies.

CHEMOENZYMATIC AND METABOLIC PROTEIN LABELING STRATEGIES

Kimberly Bonger –Chemical Immunology and Targeted Drug Delivery, Radboud University, the Netherlands
k.bonger@science.ru.nl

Enzymatic biotransformations that convert unreactive chemical moieties into reactive species have proven valuable research tools. Arylamine N-acetyltransferases (NATs) are an important class of metabolizing enzymes involved in detoxifying xenobiotic arylamines. Some NAT variants were recognized as an important factor for cancer development in humans exposed to arylamines, which is likely due to the formation of mutagenic nitrenium ions during the metabolic process. In our lab, we have adopted the unique chemoenzymatic conversion mechanism of NAT to generate nitrenium ions from aryl hydroxamic acid substrates. By varying the chemical properties of the substrates, we can tune the reactivity to other biomolecules and used NAT for imaging and mechanism-based prodrug strategies.

In the second part of the presentation, I will introduce THRONCAT, a novel metabolic labeling method based on threonine-derived non-canonical amino acid tagging. We show that the bioorthogonal threonine analog β-ethynyl serine is efficiently incorporated into NSPs, is non-toxic and allows labeling of the nascent cellular proteome in complete growth medium within minutes. To demonstrate the ease of use of THRONCAT, we profiled rapid dynamic proteomic changes of B-cells in response to an external stimulus. In addition, by combining THRONCAT with a genetic expression of a fluorescent cell marker in motor neurons, we quantified the in vivo cell type specific changes in protein synthesis rate in a Drosophila model of Charcot-Marie-Tooth peripheral neuropathy.

ORIGINS AND SIGNIFICANCE OF CHIRALITY IN SIDEROPHORES: FROM MARINE MICROBES TO PATHOGENS

Alison Butler -Department of Chemistry & Biochemistry, University of California, USA
butler@chem.ucsb.edu

Bacteria require iron to grow, yet in most environments iron is not readily available. To obtain iron, many bacteria growing aerobically produce siderophores which are ligands that bind iron(III) with high affinity and facilitate transport of Fe(III) into and within bacteria. Through genomic screening of microbes, we are identifying multiple classes of siderophores defined by variations in the chirality of amino acids. We have identified a class of tris catechol siderophores in marine and pathogenic microbes that is a variation on enterobactin, containing a combinatoric suite of D- and L- amino acids. We have also identified the origin of the diastereomers in hydroxylated amino acids that coordinate Fe(III) in peptidic siderophores. Variation in amino acid chirality affects stereochemistry at the Fe(III) site, thus affecting iron uptake in different ways. In addition to our interests in the biosynthesis of chiral centers within siderophore structures and their Fe(III) complexes, we are interested in the effects of these structural features on the process of iron uptake.

TARGETING CANCER PATHWAYS WITH CHEMICAL SWITCHES AND MOLECULAR GLUES

Arvin Dar -Department of Oncological Sciences and Pharmacological Sciences, Icahn School of Medicine, USA
arvin.dar@mssm.edu

Biological processes, such as signal transduction, gene expression, and cell proliferation, are regulated with high spatial and temporal precision. In order to study and understand these processes, equally precise conditional control is required. Small molecules and light are excellent tools for this purpose, as they can be easily regulated in timing, location, and dose, thereby enabling control of biological processes with unmatched precision. We are engineering optical and small molecule switches based on breaking covalent bonds in cells and animals to A) control nucleic acid function through synthetic installation of cleavable groups onto nucleobases and into phosphodiester backbones, and to B) control protein function through genetic code expansion with unnatural amino acids that carry protecting groups that can be removed under bioorthogonal conditions. We have applied these approaches to the conditional control of gene editing, DNA recombination, RNA polymerization, RNA translation, microRNA function, cell signaling, and other essential biological processes in mammalian cells and zebrafish embryos.

MOLECULAR SWITCHES FOR PROTEIN AND NUCLEIC ACID FUNCTION

Alexander Deiters -Department of Chemistry, University of Pittsburgh, USA
deiters@pitt.edu

Biological processes, such as signal transduction, gene expression, and cell proliferation, are regulated with high spatial and temporal precision. In order to study and understand these processes, equally precise conditional control is required. Small molecules and light are excellent tools for this purpose, as they can be easily regulated in timing, location, and dose, thereby enabling control of biological

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GENETICALLY-ENCODED CHEMISTRY IN CHEMICAL BIOLOGY

Ratmir Derda - Department of Chemistry, University of Alberta, Canada
ratmir@ualberta.ca

Genetically-encoded (GE) libraries revolutionized discovery of "biological" drugs that generate over $200 billion in sales annually. Bridging the power of genetic or DNA encoding with traditional areas of chemistry. We employ natural, GE libraries of 10^9 polypeptides made of 20 natural amino acids as a "raw material for organic synthesis". Like canonical feedstock—petroleum-derived starting materials—GE-libraries are readily available and can be transformed to useful structures through multi-step organic synthesis. "Late stage" modification of these GE-libraries in water, when optimized, can routinely convert million to billion diverse starting materials to products at once.

My talk will focus on developments from our group the use of GE-libraries of peptides as a starting material for multi-step organic synthesis to yield GE-libraries of novel macrocylic architectures that serve as promising starting point for drug discovery. We also expand generation of GE-libraries of chemicals not derived from peptides. An example is DNA-encoded "liquid glycan array (LiGA)": a reagent that encodes glycan structures and their multivalent presentation. LiGA can be combined with proteins, cells or tissues, and safely injected into animals. Simple DNA sequencing then uncovers glycan-binding preferences of said proteins, cells, tissues, or various immune cells and organs in live animals.

CONTINUOUS DIRECTED EVOLUTION OF MOLECULAR INTERACTIONS: FUNDAMENTAL STUDIES AND APPLICATIONS

Bryan Dickinson - Department of Chemical Biology, University of Chicago, Canada
dickinson@uchicago.edu

Non-covalent interactions between biomolecules such as proteins and nucleic acids coordinate all cellular processes through changes in proximity. Tools that perturb, control, or reprogram these interactions have and will continue to be highly valuable for basic and translational scientific endeavors. By taking cues from natural systems, such as the adaptive immune system, we can design directed evolution platforms that can generate proteins that bind to biomolecules of interest. Here, I will present our groups recent work toward harnessing continuous evolution platforms to study and engineer biomolecular interactions. Using our lab's split RNAP biosensing technology, I will showcase systems to evolve selective interactions between proteins, which when combined with ancestral reconstruction, and we applied to disentangle the effects of genetic background and stochasticity on evolutionary outcomes in the context of substrate binding profiles in the B-cell lymphoma-2 (BCL-2) family of apoptosis regulator proteins. Moreover, I will show vignettes using our split RNAP biosensing technology for deep-mutational scanning based on phage fitness, and for evolving "molecular glues", molecules that drive the interaction between target biomolecules. Collectively, this work highlights how advances in synthetic biology technologies that harness evolution can both shed light on how biomolecules evolve in natural contexts, as well as provide solutions to challenges in protein engineering and biotechnology.

THE CHALLENGE OF MAKING EVERY VACCINE SAFER AND MORE EFFECTIVE: MODULATING INNATE IMMUNITY VIA SIGNAL PROCESSING

Aaron Esser-Kahn- Department of Molecular Engineering, University of Chicago, USA
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The last few years have been a lesson in the power, promise and pitfalls of vaccination. Many questions remain in how both to improve the response of vaccines and to improve their tolerability. I will present novel approaches to improving both of these by controlling the signal processing within the cell using high-throughput screening to identify new molecules which work in concert with current vaccines and adjuvants.

MAKING SENSE OF SCENTS: STRUCTURAL INSIGHTS INTO ODOR DETECTION

Vanessa Franke-Ruta -, Rockefeller University/HHMI, USA
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Olfactory systems must detect and discriminate an enormous diversity of chemicals in the environment. To contend with this challenge, diverse species from humans to insects, have converged on a common strategy in which odor identity is encoded through the combinatorial activation of large families of olfactory receptors, thus allowing a finite number of receptors to detect an almost infinite chemical world. Central to this sensory coding strategy is that most individual receptors can be activated by a variety of structurally and chemically diverse odorants, suggesting that odorant detection does not adhere to the classic lock and key mechanism that governs many receptor-ligand interactions. Yet how such flexible chemical recognition has remained elusive. Our lab has been using the insect olfactory system as a powerful window into the structural logic of odor detection. In recent work, we used cryo-electron microscopy to elucidate the structure of a broadly-tuned insect olfactory receptor, MhOR5, in multiple gating states—alone, and bound to two of its ligands. These structures, along with molecular docking and functional analysis of receptor tuning, suggest that odorant recognition in MhOR5 relies predominantly on non-directional hydrophobic interactions formed with residues distributed across multiple surfaces of the binding pocket, offering a structural basis for this receptor's promiscuous chemical tuning. Our work thus begins to shed light onto the molecular recognition mechanisms that ultimately endow the olfactory system with its immense discriminatory capacity.

INFECTIOUSLY INORGANIC: A METALLOCENTRIC VIEW OF ANTIMICROBIAL ACTIVITY

Katherine J. Franz –Head of the Laboratory of Neurophysiology and Behavior, Department of Chemistry, Duke University, USA
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Normal and pathogenic cells require a menu of metal nutrients for optimal growth, but also strategies to mitigate toxicity associated with misregulated or excessive levels of metals like Fe, Cu and Zn. Cells adjust metal homeostasis mechanisms depending on cell type, local growth conditions, and in response to stress. These situations present opportunities to manipulate cellular metals as a therapeutic strategy across a number of diseases. Here I will present a metallocentric view on utilizing small molecules and peptides that leverage unique metallobiology associated with bacterial and fungal infections to selectively inhibit growth of pathogenic microorganisms. More broadly, this approach is used to explore how cellular responses at the metallomic level affect and are affected by microbial susceptibility and adaptation to antimicrobial treatment.

RIBOSWITCHING ON THE LIGHT: FLUORESCENT BIOSENSORS FOR MOLECULAR IMAGING AND DISCOVERY

Ming Chen Hammond -Department of Chemistry, University of Utah, USA
ming.hammond@utah.edu

The current decade has seen several unexpected discoveries of nucleotide-based signals that control the autonomous behavior of bacteria and immune cells. The newfound signaling pathways may provide new ways to combat antibiotic-resistant pathogens and to enhance the effectiveness of cancer immunotherapy treatments. However, critical questions remain about the biochemistry of the signaling enzymes and cell biology of the signals themselves. Tackling these questions requires tracking nucleotide-based signaling molecules in the complex environment of the cell, which poses a difficult molecular recognition challenge.

My lab has taken a structure-based design coupled to high-throughput screening approach and was among the first to develop RNA-based fluorescent biosensors, or RBF biosensors, for live cell imaging. Our biosensors exhibit remarkable specificity and affinity for nucleotide-based signals, are the brightest to date in live cell imaging studies, and can be rationally reprogrammed to sense new ligands. In this talk, I will present the design principles that enable effective allosteric coupling of ligand binding to fluorescence activation of a small molecule chromophore. We have demonstrated performing, in essence, in vivo biochemistry experiments to track dynamic effects of endogenous chemical cues and inhibitor compounds on enzyme activity in live bacteria. This approach has revealed a new strategy to combat antibiotic resistance. We also have applied these biosensors to make several biological discoveries, including a signaling pathway that regulates how some bacteria interact with redox reactive surfaces. Finally, I will describe another broad application for these biosensors, as novel high-throughput screening assays for enzyme discovery.

THE INS AND OUTS OF INITIATING GLYCOCONJUGATE BIOSYNTHESIS AT THE MEMBRANE FRONTIER

Barbara Imperiali -Department of Biology and Chemistry, Massachusetts Institute of Technology, USA
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Complex glycoconjugates serve critical functions across domains of life. In bacteria, diverse biomolecules, such as the bacterial peptidoglycan and capsular polysaccharide afford mechanical stability to unicellular organisms in rapidly changing environments and often act as virulence factors. Complex glycolipids, including the O-antigen component of the lipopolysaccharide (LPS) of Gram-negative bacteria, function to mediate interactions amongst cells and propagate deleterious pathogenic processes and, glycoproteins in prokaryotes and eukaryotes are important cell-surface determinants involved in myriad cellular functions.

The biosynthesis of a majority of glyconjugates occurs via an en bloc mechanism involving sequential stepwise glycan assembly onto a polyprenol phosphate-linked carrier at cellular membranes including the inner membrane of Gram-negative bacteria and the ER membrane of eukaryotes. This presentation focuses on the initial membrane-committed step of the biosynthetic pathways that is catalyzed by phosphoglycosyl transferases (PGTs). PGTs catalyze the transfer of a phospho-sugar from a soluble nucleoside diphospho-sugar to a membrane-resident polyprenol phosphate. Studies on the PGTs have been hampered because they are integral membrane proteins, and often prove to be recalcitrant to expression, purification, and analysis. However, in recent years exciting new information has been derived on the structures and the mechanisms of PGTs, revealing two unique superfamilies of PGT enzymes that catalyze phosphoglycosyl transfer at the membrane interface. Genome neighborhood analysis shows that these superfamilies, the polytopic PGT (polyPGT) and monotopic PGT (monoPGT), may initiate different pathways within the same organism. Moreover, the same fundamental two-substrate reaction is enacted through two distinct chemical mechanisms with differing modes of catalysis. This presentation highlights the structural and mechanistic divergence between the PGT

enzyme superfamilies and how this is reflected in differences in regulation in their varied glycoconjugate biosynthesis pathways.

TOTAL SYNTHESIS OF INNATE IMMUNE FUNCTIONS IN INERT AND ARTIFICIAL CELLS

Takanari Inoue -Department of Cell Biology, John Hopkins Medicine, USA
jctinoue@jhmi.edu

Molecular events driving dynamic innate immune functions such as chemotaxis, phagocytosis and degranulation are localized and rapid, often complicated by the presence of feedback loops and crosstalk at the level of signal transduction. Conventional tools used to probe cell signaling are often limited due to its speed and global effect. My scientific research career, to date, has focused on the development of alternative visualization and perturbation strategies for deconstructing spatiotemporally dynamic signaling events observed in nature, with a specific focus on immune functions of neutrophils, macrophages, and mast cells. These strategies include series of molecular sensors and actuators based on chemically-induced dimerization techniques that allow for the induction of specific activity at different subcellular localizations of live cells in the order of seconds. We have recently extended these techniques to reconstitute these cellular functions in non-immune cells, and even in artificial cells. Ultimately, we will employ the knowledge and skills acquired from these synthetic studies on the complex cell behaviors to endow cells with important therapeutic functions with immediate applications in the treatment of cancers, neurodegenerative diseases and allergies.

NEW TOOLS FOR MONITORING & MANIPULATING CELLULAR ACTIVITY

Loren Looger – HHMI, Univ. California San Diego, USA
loogerl@janelia.hhmi.org

I will present updates on a variety of molecular tools for studying & manipulating neural circuits & other preparations. Topics include genetically encoded calcium indicators (including the new ultra-fast jGCaMP8 variants), neurotransmitter sensors (improved versions for following glutamate, GABA, acetylcholine, serotonin), optogenetic effectors including the new "enhanced Magnets" dimerizers, AAV serotypes for retrograde labeling & altered tropism, probes for correlative light-electron microscopy, chemical gene switches, etc. These seminars are typically very interactive, including audience members choosing the topics covered. I will make all my slides freely available - so don't worry about hurriedly taking notes; instead focus on questions and ideas for collaboration. Please bring your suggestions for molecular tools that would be transformative for the field.

CHEMICAL BIOLOGY OF THE INNATE IMMUNE STING PATHWAY

Lingyin Li -Department of Biochemistry, Stanford University, USA
lingyinl@stanford.edu

The 2018 Nobel Prize in Medicine honored the discovery of cancer therapy that blocks adaptive immune checkpoints. These therapies are now curing ~20% of previously considered terminally ill melanoma patients because melanomas are immunogeneic tumors heavily infiltrated by immune cells (also known as “hot tumors”). However, they have not been effective in most cancers which are less immunogenic (also known as “cold tumors”). The innate immune STING (stimulator of interferon genes) pathway is now recognized as a central pathway in anti-cancer immunity because it senses cytosolic double-stranded DNA (dsDNA), a hallmark of cancers due to their frequent erroneous chromosomal segregation. Upon sensing of cytosolic dsDNA, cyclic-GMP-AMP-synthase (cGAS) produces the second messenger 2’3’-cyclic- GMP-AMP (cGAMP) in the cytosol, which binds to and activates its receptor STING. STING activation leads to production of type-I interferons, a potent cytokine that then activates downstream immune responses aginst cancer. My lab has identified the role of cGAMP

as an immunotransmitter that alerts our immune system of cancer. We have also uncovered regulation mechanisms of extracellular cGAMP signaling including how it activates STING and its degradation enzyme and transporters. We identified ENPP1, the dominant hydrolase of cGAMP, as an innate immune checkpoint and important target for cancer immunotherapy. We developed promising drug candidates that one day may benefit patients with cold tumors that previously do not respond to adaptive immune checkpoint blockers.

COORDINATION OF CORTICAL ACTIN AND ER-PM CONTACT SITES DIRECTS RECEPTOR SIGNALING TO THE FRONT DURING CELL MIGRATION

Tobias Meyer -Departments of Biochemistry and Cell & Developmental Biology, Weill-Cornell Medical College, USA
tom4003@med.cornell.edu

Bo Gong, Alex Thiemicke and Tobias Meyer, Weill Cornell Medicine, NY

My laboratory is interested in understanding how mammalian cells integrate signals to control cell function. A particularly fascinating problem we are focusing on is the interplay between signaling polarity and the polarity of membranes and actin organization during directed cell migration. My talk will focus on the question how the cortical actin network and ER-PM contact sites direct receptor tyrosine kinase signaling to the front which in turn directs membrane protrusions during cell migration. I will discuss the use of a reporter system that we developed to measure the local density of F-actin close to the plasma membrane along with the dynamic organization of ER-PM contact sites in migrating cells. We identified a parallel gradient of both membrane proximal actin and ER-PM contact sites, being high in the back and low in front. A main purpose of the gradient of membrane proximal F-actin is to increase Rac signaling to the front to both direct local protrusions and stabilize polarity and ensure that cells persistently migrate. A main purpose of the gradient in ER-PM contact sites is to localize EGF receptor activity to the front by increasing the rate of dephosphorylation of EGFR by PTB1B phosphatase in the back. PTB1B activity is localized to the back since the density of ER-PM contact sites is much higher there compared to the front and the exclusively ER localized PTB1B can only interact with EGFR's at ER-PM contact sites. Overall, our findings argue that the polarization of signaling in cell migration results from a close interplay between the spatial organization of cortical actin and ER-PM contact sites, along with feedback mechanisms that orient receptor, lipid second messengers and small GTPase signaling.

BUILDING MOLECULAR COMPLEXITY THROUGH THE REACTIONS OF VINYL CARBOCATIONS AND THE APPLICATIONS OF ELECTRON DIFFRACTION IN THE CHARACTERIZATION OF COMPLEX MOLECULES.

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In this talk I will discuss our recent efforts to utilize phenyl and vinyl carbocations in C–H functionalization reactions. We will describe how these high-energy dicoordinated carbocations can be generated under mild conditions and utilized in the selective C–C bond forming reactions of simple hydrocarbons. Moreover, we will discuss our efforts to understand the mechanism of these reactions through computational chemistry, kinetics, electron microscopy, and isotopic labeling studies. In the second half of the talk we will discuss our efforts to bring electron microscopy to organic chemistry through the use of MicroED and other CryoEM modalities.

CHEMOGENETICS FOR RESEARCH AND POTENTIAL TRANSLATIONAL APPLICATIONS

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Localized control of neuron activity is important for both brain research and therapy. Chemogenetics is a method to control cellular activity by targeting defined cell populations with an exogenous receptor that is engineered to respond selectively to a small-molecule agonist. The approach is generalizable because a receptor-agonist combination can be used to activate or inhibit different neural populations in any brain region. Moreover, using agonists that are selective for the chemogenetic receptor allows cell type–specific modulation, in contrast to traditional pharmacology. Chemogenetic tools have achieved widespread utility in animal models, and there is growing interest in developing chemogenetic systems that are suitable for human therapeutic applications. We developed a toolbox of modular ion channels and selective, ultrapotent agonists that can be used for targeted control of brain activity in rodent and primate models. Additional studies will be needed to establish long-term safety and efficacy with chemogenetic receptors for therapeutic applications, but this is facilitated by using FDA-approved drugs as chemogenetic agonists. These chemogenetic technologies can advance research into neural circuit disorders while enabling extension to human therapies.

DISPLAY OF PSEUD-NATURAL PEPTIDES AND PRODUCTS

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Macrocyclic peptides possess a number of pharmacological characteristics distinct from other well established therapeutic molecular classes, resulting in a versatile drug modality with a unique profile of advantages. Macrocyclic peptides are accessible by not only chemical synthesis but also ribosomal synthesis. Particularly, recent inventions of the genetic code reprogramming integrated with an in vitro display format, referred to as RaPID (Random non-standard Peptides Integrated Discovery) system, have enabled us to screen mass libraries (>1 trillion members) of non-standard peptides containing multiple non-proteinogenic amino acids, giving unique properties of peptides distinct from conventional peptides, e.g. greater proteolytic stability, higher affinity (low nM to sub nM dissociation constants similar to antibodies), and superior pharmacokinetics. The field is rapidly growing evidenced by increasing interests from industrial sectors, including small start-ups as well as mega-pharmas, toward drug development efforts on macrocyclic peptides, which has led to several *de novo* discovered peptides entering clinical trials. This lecture discusses the aforementioned screening technology, the RaPID system, and several showcases of therapeutic potentials of macrocyclic peptides. This lecture also discusses the most recent advance in the display of pseudo-natural products generated by thiopeptide post-translationally modifying enzymes.

"Accurate Broadcasting of Substrate Fitness for Lactazole Biosynthetic Pathway from Reactivity-Profiling mRNA Display." A. Vinogradov; E. Nagai; J.S. Chang; K. Narumi; H. Onaka; Y. Goto; H. Suga*; **Journal of the American Chemical Society**, (2020)

"Ribosomal Elongation of Aminobenzoic Acid Derivatives." T. Katoh; H. Suga*; **Journal of the American Chemical Society**, 142, 16518-16522 (2020)

"GTP-State-Selective Cyclic Peptide Ligands of K-Ras(G12D) Block Its Interaction with Raf." Z. Zhang; R. Gao; Q. Hu; H. Peacock; D.M. Peacock; S. Dai; K.M. Shokat; H. Suga* **ACS Cent. Sci.**,(2020)

"Development of cyclic peptides with potent in vivo osteogenic activity through RaPID-based affinity maturation" N. K. Bashiruddin; M. Hayashi; M. Nagano; Y. Wu; Y. Matsunaga; J. Takagi; T. Nakashima; H. Suga*; **Proceedings of the National Academy of Sciences USA** 117(49)31070-31077(2020)

"Ribosomal synthesis and de novo discovery of bioactive foldamer peptides containing cyclic β-amino acids" T. Katoh; T. Sengoku; K. Hirata; K. Ogata; H. Suga*; Nature Chemistry 12, 1081-1088 (2020)

"Promiscuous enzymes cooperate at the substrate level en route to lactazole A" A.A. Vinogradov; M. Shimomura; N. Kano; Y. Goto; H. Onaka, H. Suga*; **Journal of the American Chemical Society** 142, 13886-13897 (2020)

"Introduction to Thiopeptides: Biological Activity, Biosynthesis, and Strategies for Functional Reprogramming" A.A. Vinogradov; H. Suga*; **Cell Chemical Biology**, Accepted article (2020)

"Macrocyclic Peptide-Mediated Blockade of the CD47-SIRPα Interaction as a Potential Cancer Immunotherapy" D. Hanzawa; Y. Yin; Y. Murata; M. Matsuda; T. Okamoto; D. Tanaka, N. Terasaka; Jinxuan Zhao, M. Sakamoto;

Y. Kakuchi; Y. Saito; T. Kotani; Y. Nishimura; A. Nakagawa; H. Suga*; T. Matozaki; **Cell Chemical Biology**, 27, 11811191 (2020)
"Macrocyclic peptide-based inhibition and imaging of hepatocyte growth factor K." Sakai; T. Passioura; H. Sato; K. Ito; H. Furuhashi; M. Umitsu; J. Takagi; Y. Kato; H. Mukai; S.Warashina; M.Zouda; Y.Watanabe; S.Yano; M.Shibata; H. Suga*; K. Matsumoto; **Nature Chemical Biology**, 15, 598-606 (2019)
"De novo macrocyclic peptides that specifically modulate Lys48-linked ubiquitin chains." M. Nawatha; J.M. Rogers; S.M. Bonn; I. Livneh; B. Lemma; S.M. Mali; G.B. Vamisetti; H. Sun; B. Bercovich; Y. Huang; A. Ciechanover; D. Fushman; H. Suga*; A. Brik; **Nature Chemistry**, 11(7):644-652 (2019)

BIOCONJUGATE STRATEGIES TO TARGET THE IRON CORE OF CANCER

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The central role of iron in tumor progression and metastasis motivates the study of iron-binding compounds, such as thiosemicarbazone and aroylhydrazone chelators, as anticancer therapeutic candidates. These approaches affect both malignant cells and tumor-associated macrophages, thereby targeting multiple iron-dependent programs that support cancer growth. Disulfide-based prochelators sequester intracellular iron because they are activated for iron coordination upon cellular uptake and reduction in the presence of elevated levels of glutathione. Herein, we report on the synthesis and characterization of bioconjugate prochelator systems designed to increase cancer selectivity. In a glycoconjugation strategy, disulfide linkages connect iron-binding units to carbohydrate moieties that exploit the avid glucose consumption of cancer cells (Warburg effect). We find that the glycoconjugate systems rely, at least in part, on glucose transporter GLUT1 for cellular uptake and that their antiproliferative activity is reduced in the presence of an inhibitor that blocks the transporter. In isogenic cell pairs, in which one cell line has been transduced to amplify GLUT1 expression, the IC50 values of the glycoconjugate prochelators clearly correlate with GLUT1 expression (whereas those of aglycone controls remain unchanged). These findings indicate that glycoconjugation is a viable strategy to target malignant cells exhibiting high glucose consumption. Within this study of disulfide-based prochelators, we also found that compounds featuring alkylated thiosemicarbazone and imidazole-2-thione moieties react with serum albumin, which is a major component of cell growth media and the most abundant protein in human blood. Native mass spectrometry experiments demonstrated a covalent modification of the protein consistent with thiol-disulfide exchange involving a reduced cysteine residue. In a panel of breast, ovarian and colorectal cancer cell lines, the constructs present antiproliferative activities at submicromolar concentrations □ the best activity profiles within their class of disulfide-based prochelators. These prochelators therefore combine a promising toxicity profile with albumin bioconjugation, which could confer enhanced lifetime in blood circulation and tumor accumulation.

CHEMICAL-PROTEOMIC STRATEGIES TO INVESTIGATE REACTIVE CYSTEINES

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Cysteine residues are critical to the catalytic and regulatory functions of diverse proteins including proteases, oxidoreductases and kinases. Although the majority of catalytic cysteine residues are well annotated, the identity and endogenous functions of regulatory and metal-binding cysteines are poorly understood. Regulatory cysteines are often located distal to the catalytic or ligand-binding sites of proteins and regulate protein function through posttranslational modifications, such as oxidation. Metal-binding cysteines provide coordination to a variety of metal ions, including zinc and iron, and are essential to maintaining the structure and function of diverse metalloproteins. Oxidation and coordination to metal ions result in distinct changes in cysteine reactivity, and we have developed a suite of chemoproteomic approaches for monitoring these cysteine-reactivity changes directly in cell lysates and living cells. Our efforts have generated a suite of chemical probes to investigate cysteine function, and unearthed previously uncharacterized functional cysteines implicated in catalysis and regulation.

DISCOVERY OF A DEGRON FOR THE THALIDOMIDE BINDING DOMAIN OF CEREBLON

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The E3 ligase adaptor cereblon is a target of thalidomide and lenalidomide, therapeutic agents that are used in the treatment of hematopoietic cancers despite teratogenic toxicity. These agents act in part by modulating substrate selection and degradation through the thalidomide binding domain of cereblon. However, despite the expanding use of cereblon in targeted protein degradation technologies, identification of a degron that controls the endogenous substrate selection mechanisms of cereblon has remained elusive. Here, I will describe chemoproteomics approaches to target identification in the study of molecular glues like lenalidomide, and how these chemical biology approaches led to the discovery of a degron for the thalidomide binding domain of cereblon.

#111 - NEW PHARMACOLOGICAL STRATEGIES FOR TUNING G PROTEIN SIGNALING

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The G protein-coupled receptor (GPCR) cascade leading to production of the second messenger cAMP is replete with pharmacologically targetable receptors and enzymes with the exception of the stimulatory G protein α subunit, Gαs. GTPases remain largely undruggable given the difficulty of displacing high affinity guanine nucleotides and the lack of other drug binding sites. We explored a chemical library of 10^12 cyclic peptides in order to expand the chemical search for inhibitors of this enzyme class. We identified two macrocyclic peptides, GN13 and GD20, that antagonize the active and inactive state of Gαs, respectively. Both GN13 and GD20 showed high G protein specificity and nucleotide-binding-state selectivity. Co-crystal structures reveal that GN13 and GD20 distinguish the conformation difference within the switch II / α3 pocket in Gαs and directly block effector interactions. GN13 and GD20 modulate Gαs function, including Gαs steady state GTPase activity, Gαs-mediated adenylyl cyclase activation, and Gαs-Gβγ interaction, through binding to the crystallographically defined pocket. Intriguingly, the active state inhibitor GN13 and its analog GR6_F2Y potently inhibit constitutively activated oncogenic mutants of Gαs (Q227L and R201C), which suggests a promising therapeutic approach for Gαs-driven cancer. The discovery of conformation-selective cyclic peptide inhibitors targeting Gαs provides path for the development of state-dependent GTPase inhibitors.

#169 - CHEMICALLY ACTIVATED PROXIMITY LABELING WITH BIOORTHOGONAL PHOSPHINES

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Protein-protein and other biomolecule interactions drive fundamental cellular processes, but the timing and location of these interactions remain poorly understood. This is due, in part, to a lack of reliable probes for capturing biomolecule associations in native environments. Current strategies require intense light or oxidants, which can limit applications in live cells and tissue. We aimed to develop a more general, biocompatible approach to map biomolecule interactions. Toward this end, we coopted bioorthogonal phosphines and cyclopropenones (CpOs) for proximity labeling. CpOs can be selectively triggered by phosphine reagents to covalently tag neighboring biomolecules, marking them for visualization and identification. We synthesized a variety of CpO analogs and characterized their reactivity patterns in vitro. We further applied the bioorthogonal platform to monitor lipid interactions in live cells. Collectively, these studies showcase the versatility of chemically triggered CpOs for examining biomolecular networks.

#121 - EXPLORING THE ROLES OF HOST LIPIDS DURING SARS-COV-2 INFECTION

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Positive-stranded RNA viruses, including flaviviruses and coronaviruses, produce dramatic membrane rearrangements within their host cells. SARS-CoV-2 produces a characteristic array of double-membraned vesicles, thought to be the site of viral replication, and convoluted membranes, with less well-defined functions. Following results in flaviviruses, notably ZIKV, we hypothesized that this dramatic program of membrane remodeling would require a corresponding remodeling of the host lipid composition and substantially perturb cellular lipid-protein interactions. To assess this, we performed global non-targeted lipidomics on HEK-293T cells infected with SARS-CoV-2, and further performed global non-targeted lipidomics on HEK-293T cells transfected with 24 of the 29 SARS-CoV-2 proteins. We found that SARS-CoV-2 dramatically alters the host lipid landscape, increasing the abundance of host

TAG species up to 64-fold, inducing the production of ceramide, and increasing the number of polyunsaturated phospholipid species while decreasing the number of saturated phospholipid species. We then correlated these lipidomic changes with cellular phenotypes, including the induction of lipid droplets, and showed that inhibition of key lipid biosynthetic enzymes prevented viral replication. Finally, we showed that SARS-CoV-2 alters the subcellular location of specific lipid species, especially sphingolipids, using synthetic trifunctional lipid probes. These observations demonstrate the intimate relationship between SARS-CoV-2 and the lipids of its eukaryotic host, which is complex, critical for viral success, and vulnerable to host-targeted therapeutics. By analyzing the lipid interactomes with the help of multifunctional lipid derivatives, we will identify viral and host cell targets in the future.

#147 - Developing Top-down Mass Spectrometry Methods for the Investigation of Genetically Engineered Protein Systems

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Mass spectrometry (MS) is an extremely sensitive tool that can be used for protein sequencing and localization of post translational modifications (PTMs). Although the status quo for protein sequencing historically involves an enzymatic digest before analysis, recent advancements in MS fragmentation technology have enabled “top-down” analysis which uses in-tact proteins for sequencing. Electron capture dissociation (ECD) is an advantageous MS fragmentation technique because it enables efficient protein sequencing while retaining sensitive PTMs such as phosphorylation. Our group develops a tool called an ExD cell, which enables ECD fragmentation in commonly available mass spectrometers. The past 2 years have demonstrated the critical need for research on SARS-CoV-2 virus. In collaboration with The Unnatural Protein Facility at Oregon State University, we recently applied our top-down MS methods to study two important virus-related proteins. This publication will first describe the use of ECD-MS to map sequential phosphorylation of the SARS-CoV-2 nucleocapsid (N) by host enzyme GSK-3β. Then, we describe a second project where ECD-MS was used to characterize a potential anti-viral in the form of a spike (S) protein nanobody. The SARS-CoV-2 N protein plays a key role in repackaging RNA into new viral particles. The N protein mechanism is tightly regulated by sequential phosphorylation of a serine and arginine (SR)-rich region. Host enzyme GSK-3β has been identified as the primary regulator of phosphorylation in the SR-rich region, however, the extent of phosphorylation by GSK-3β has not been mapped with precision using MS. The gap in characterization of the SR-rich region results from two major analytical challenges. First, GSK-3β requires a site-specific ‘primer’ phosphorylation that enables kinase docking and subsequent phosphorylation. Second, SR-rich proteins are notoriously poor candidates for common MS workflows involving enzymatic digests due to repeating sequences containing tryptic cleavage sites. To solve these issues, we used genetically encoded phosphorylation to serve as ‘primers’ for GSK-3β. Then, the ‘primed’ SR-rich proteins were reacted with GSK-3β, and the resulting phosphorylation patterns were analyzed using top-down ECD-MS. Top-down MS enables the measurement of intact protein populations, protein sequence, and PTM localization within one experiment. Using these methods for the N protein, we found that there were several populations of the phosphorylated N protein containing 4-6 phosphorylations depending on the site of the genetically encoded ‘primer’. ECD fragmentation was used to localize each site of phosphorylation resulting from the genetically encoded ‘primer’ modifications. In the second project, we applied our top-down methods again to characterize a SARS-CoV-2 nanobody (Nb) that could be customized to be a monomer, dimer, or trimer. This provides significant advantage because the Nb targets the trimeric S protein, therefore, targeting each subunit with a trimeric Nb may be more effective at blocking the virus. In our analysis, we were able to confirm the identity of the Nb monomer and the PEG-conjugated dimer and timers. ECD-fragmentation was used to confirm the site of a genetically encoded tetrazine amino acid which enabled the

chemistry that conjugated the Nb monomers together through a PEG moiety. Combining top-down ECD-MS with genetic engineering provides new opportunities for studying protein PTMs and can improve the way we make and characterize protein therapeutics. In our recent efforts we have explored the synergy between top-down MS and genetic engineering by mapping new phosphorylation patterns in the N protein SR-rich region and by characterizing the flexible topology of the spike protein Nb. With the rising popularity of protein engineering techniques combined with advances in analytical tool such as ECD-MS, the future partnership of these methods looks bright for protein research.

#124 - NEW THERAPEUTIC PLATFORMS FOR TARGETING METAL NUTRIENT HOMEOSTASIS VULNERABILITIES IN CANCER

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Aberrant cell proliferation in cancer is highly dependent on nutrient status, including transition metals like copper, iron, and zinc. In particular, while copper is an essential nutrient for all cells, tumor cells display a heightened requirement for this metal and elevated expression of metallochaperones that regulate its homeostasis. Indeed, high levels of Atox1, a canonical copper metallochaperone, correlates with poor patient prognosis in melanoma and breast cancer, highlighting its value as a novel therapeutic target for cancer. We present the development of a therapeutic platform for identifying and drugging copper-dependent targets in cancer using activity-based protein profiling (ABPP). This chemoproteomics method enables high-throughput, unbiased discovery of new covalent ligands to engage and functionally modulate Atox1, establishing a generalizable approach to target a broader range of metal-dependent disease vulnerabilities.

#141 - PHOTOACTIVATED PROTEOLYSIS TARGETING CHIMERAS (PHOTACS) FOR LIGHT-ACTIVATED DEGRADATION OF SYNAPTIC PROTEIN CAMKII IN HIPPOCAMPAL TISSUE

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Proteins control a wide range of cellular and biological functions. To study the role of such proteins, gene knockout strategies are generally employed but the results are often obscured by compensation from other proteins or perinatal lethality. PHOTACs (PHOtochemically TArgeting Chimeras) are photoswitchable bifunctional molecules that can, with the spatiotemporal precision of light, target proteins for ubiquitination by an E3 ligase complex and subsequent degradation by the proteosome. Here we demonstrate a strategy to selectively and reversibly, with the precision of light, control the synaptic function of CAMKII (Ca2+ /calmodulin-dependent protein kinase II) utilizing a PHOTAC molecule in wild-type mice brain tissue. Our modular approach provides a novel and powerful method to study the function of proteins in native systems that could lead to the discovery of new functions for many other proteins.

#117 - MULTIFUNCTIONAL FLUOROPHORES AS MOLECULAR TOOLS BEYOND IMAGING

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Small molecules are vital to biology and medicine. For example, 60% of drugs are small molecules. However, controlling the permeability of small molecules across the cellular membrane to reach their protein targets is still challenging. Further, strategies to visualize them within the cellular environment by tagging them to fluorescent dyes often result in further loss of permeability of the dye–small-molecule conjugate and small molecule's activity. Strategies to obtain such conjugates to visualize and manipulate biomolecules will advance our understanding of cellular processes. Recent improvements in the photophysics and bioavailability of organic dyes combined with their deployment with genetic specificity via self-labeling tags (e.g., HaloTag) have enabled new imaging experiments in living systems. However, dyes that facilitate additional functions (e.g., quantification of ions, recruitment of enzymes) on labeled proteins via a small-molecule cargo are rare. I have developed multifunctional dyes that leverage the brightness, fluorogenicity (i.e., non-fluorescent to fluorescent), and tunable cell permeability of far-red emitting Janelia Fluor (JF) dyes to push their utility from solely imaging labeled proteins to also manipulating them via the dye-linked cargo (e.g., affinity tags, pharmacologics). These multifunctional dyes have three components working in unison: genetic targeting ligand for specific labeling of protein of interest; bright and photostable JF dye for fluorescence readout; and small-molecule cargo for manipulating the labeled biomolecule. We demonstrate the potential of multifunctional dyes for applications beyond imaging using two novel reagents: cell-membrane permeable biotin-dye for affinity purification of intracellular proteins; and cell- and nuclear-membrane permeable JQ1-dye for recruiting Brd4 from heterochromatin to labeled proteins in euchromatin. Further, we show that this strategy is easily extendable to other biomolecules such as DNA. Here, we show that JQ1-polyamide-dye allows the recruitment of Brd4 to specific DNA sequences for increased transcription. In conclusion, multifunctional dyes allow control over cell permeability while maintaining targetability, fluorescence, and a functional cargo to enable superior tools for cell biology, pharmacology, and advanced imaging, thereby representing a new avenue in the development of dye-based tools.

#174 - REGULATION OF CALCIUM OSCILLATIONS IN B-CELLS BY CO-ACTIVATED CANNABINOID RECEPTORS

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Pharmacological treatment of pancreatic β cells targeting cannabinoid receptors 1 and 2 (CB1 and CB2) has been shown to result in significant effects on insulin release, possibly by modulating intracellular calcium levels ([Ca2+]i). It is unclear how the interplay of CB1 and CB2 affects insulin secretion. Here, we demonstrate by live cell intracellular calcium imaging and the use of highly specific receptor antagonists as well as a recently developed photo-releasable endocannabinoid 2-arachidonoylglycerol that both receptors have counteracting effects on cytosolic calcium oscillations. We further show that both receptors are juxtaposed in a way that increases [Ca2+]i oscillations in silent β cells but dampens them in active ones. This study highlights a functional role of CB1 and CB2 acting in concert as a compensator/attenuator switch for regulating β cell excitability.

#199 - VARIATIONS IN NUCLEOLAR STRESS PRODUCED BY SMALL-MOLECULE PT(II) COMPOUNDS

Pigg, H. C., Yglesias, M. V., Sutton, E. C., McDevitt, C. E., Guerrero, A. S., Shaw, M. and DeRose, V. J., 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 oxalipilatin and related Pt(II) compound derivatives induce nucleolar stress. Structure-function analyses with focus on the diaminocyclohexane (DACH) carrier ligand of oxalipilatin have discovered a limited number of derivatives that cause nucleolar stress in A549 lung cancer cells. These compounds vary in the initial induction time of nucleolar stress, as well as the overall degree of stress induced. Both the ring size and stereochemistry of the non-aquation-labile ligand influence nucleolar stress induction. We observe that Pt(II) compounds containing a 6 membered non-aquation-labile ring show faster onset and a higher overall degree of nucleolar stress than those containing a 5-membered ring. Additionally, compounds having the 1R,2R stereoisomeric conformation show faster onset and a higher overall degree of nucleolar stress than those having the 1S,2S conformation. The degree of nucleolar stress induction does not correlate with either cellular uptake or level of cellular Pt(II)-DNA adduct formation, indicating a more specific influence of the Pt compounds on nucleolar processes. 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.

#109 - ZINC SIGNALLING AND METAL ION HOMEOSTASIS IN MAMMALIAN CELL CYCLE

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Zinc (Zn) is a quintessential micronutrient which plays significant roles in cellular growth and proliferation.1,2 At the cellular level, zinc homeostasis and zinc dependent biological processes are regulated by a plethora of well-coordinated transcription factors, genes, and proteins.3-5 However, unravelling the intricate detail of mechanistic pathways by which zinc participates in regulation of cell proliferation is a challenging task to address. Previous studies on zinc perturbation and cell proliferation have revealed that zinc deficiency induces a cell cycle arrest state (quiescence state) in asynchronously cycling mammalian cells and those quiescent cells showed significant changes in other biologically relevant metal ions levels.2 Those observations motivated us to ask questions such as: 1. What are the forefront players responsible for the zinc deficiency (ZD) induced quiescence state; 2. How are other metals changed in ZD induced quiescence cells. To decipher the effect of zinc perturbation on the mammalian cell cycle, we used the approach of knocking down Zn2+ regulatory proteins and transcription factors with a focus on knockdown of Zinc responsive metal regulatory transcription factor-1 (MTF-1). Knockdown of MTF1 is expected to alter expression of downstream target genes in response to zinc. This includes proteins such as metallothionin (MT) and ZnT1 that are responsible for sequestering and exporting high level of labile Zn2+ pool to maintain zinc homeostasis, respectively. After successfully establishing a knockdown (MTF1 KD) MCF10A cell line and a scrambled control (Scr-Ctrl) cell line, we explored how the KD cells would respond to variable cytosolic zinc concentrations. A cell proliferation assay on cells with zinc deficient and zinc enriched (ZR) conditions for 48h revealed that while the Scr-Ctrl cells thrived under 50ZR (50 μM of ZnCl2 in minimal media(MM)), knockdown cells struggled to proliferate. Proliferation of the KD cell line is significantly lower compared to MM condition and to Scr-Ctrl cells treated with MM and 50ZR condition. This result was again confirmed by fluorescence image analysis from long term imaging of cells for 60h under different zinc conditions. Treatment with zinc-rich media on Scr-ctrl and KD cells showed that the downstream expression level of MT is not induced in KD cells compared to Scr-ctrl cells. This reinforced our understanding that knockdown cells are unable to regulate the zinc homeostasis and which directly affects the proliferation of KD cells. We are also trying to address how different metal ions cross-talk with each other in the quiescence state and whether there is a correlation between different metal ion levels and differentially expressed metallo-regulatory genes in quiescent cells. Recent results and our understandings would be presented in detail in the conference.

#150 - Se EXAFS reveals direct substrate binding function for auxiliary iron-sulfur clusters in radical-SAM catalyzed thioether crosslinking

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Site-specific selenium (Se) substitution of mechanistically relevant sulfur atoms is a powerful X-ray spectroscopic probe in investigations of metalloprotein active sites. We have used Se substitution to study the mode of substrate binding in two radical SAM enzymes which catalyze thioether crosslink formation in target ribosomally translated peptides (RiPPS). In addition to the radical SAM cluster, both enzymes contain two auxiliary iron-sulfur (Fe-S) clusters of unknown function in a motif known as a SPASM domain. These Fe-S cofactors have been demonstrated to be required for intramolecular thioether formation between a cysteine (Cys) thiolate and carbon atom activated by the radical SAM-generated dAdo radical on the substrate peptide. To test the hypothesis that the Cys residue undergoing thioether formation coordinates directly to one of the auxiliary clusters, we replaced the peptide substrate Cys residue with selenocysteine (SeCys). Se K-edge EXAFS analysis of this peptide in the presence of enzyme revealed a direct Se-Fe interaction as the mode of selenosubstrate binding and mass spectrometry data have validated that the SeCys-substituted substrate forms the same chalcogenoether product as the native Cys-containing substrate. A second enzyme system amenable to Fe-S cluster deletion variants allowed us to assign auxiliary cluster 1 as the site of SeCys substrate binding – this Fe-S cluster is site-differentiated with an open coordination position, which we reasoned was the most likely location for substrate selenolate coordination. This work has assigned a direct substrate binding function for auxiliary cluster 1, which will help inform future mechanistic work on SPASM-domain containing peptide maturase systems. More broadly, we are excited about this application of Se substitution to thiolates of interest in radical SAM transformations of cysteine substrates, which would be otherwise difficult to observe in the selected sulfur-rich systems.

#119 - Bio-orthogonal Noncanonical Amino acid Tagging Reveals Novel interspecies Antagonism

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Tirrell, D. A., California Institute of Technology

Chronic infection by polymicrobial communities is the leading cause of morbidity and mortality for people with cystic fibrosis (CF). Interspecies interplay between Pseudomonas aeruginosa and Staphylococcus aureus—the two most prevalent species in the CF lung microbiome—critically affects CF disease progression as well as patient response to antibiotic treatment. In the present study, we used Bioorthogonal Non-canonical Amino acid Tagging (BONCAT) to investigate interspecies dynamics between the two opportunistic pathogens predominating CF infection. P. aeruginosa and S. aureus cells were engineered to express a mutant tRNA synthetase that allows for metabolic labeling of newly synthesized proteins by azide-bearing non-canonical amino acids, enabling time-resolved and cell-selective interrogation of global proteomic fluctuations in both species. First, we show that P. aeruginosa robustly outcompete S. aureus even when comprising only 10% of the total starting population. We further demonstrate that cell-selective BONCAT afforded ~12-fold enrichment of proteins synthesized by S. aureus in a co-culture environment highly predominated by P. aeruginosa, highlighting BONCAT as a powerful chemo-selective strategy that facilitates targeted proteomic analysis of low-abundance organisms in polymicrobial systems. Moreover, using time-resolved BONCAT to measure the immediate global proteomic response of P. aeruginosa in co-culture with Methicillin-resistant Staphylococcus aureus (MRSA) USA300, we discovered previously undescribed systematic induction of the Type VI secretion system (T6SS). Despite an abundance of existing body of work analyzing differential gene expressions for P. aeruginosa

in co-culture, our study is, to the best of our knowledge, the very first to report T6SS activation in P. aeruginosa in response to gram-positive bacteria. In summary, the distinct cell-selectivity and precise temporal control of analysis of protein synthesis demonstrated in our study presents BONCAT proteomics as a state-of-the-art physiology approach for unraveling novel biology at the host-pathogen and polymicrobial interactions interface.

#120 - LIPID EXPANSION MICROSCOPY

White-Mathieu, B., Conwell, M., Wu, K., and Baskin, J., Cornell University

Membrane architectures whose dimensions and features are smaller than the diffraction limit of light orchestrate diverse cellular events such as lipid transport, vesicle formation, and calcium signaling. These structures, which include membrane invaginations, organelle contact sites, and membrane microdomains, are primarily composed of phospholipids, making methods to visualize these biomolecules vital to our understanding of cellular function. Techniques to accurately image phospholipid-containing structures with fluorescence microscopy are challenged by the diffusion of lipids within the bilayer, even in fixed samples. Expansion microscopy (ExM) utilizes hydrogel formation to fix biomolecules in place and swell samples to produce high-resolution images of protein- and nucleic acid-containing cellular structures 30-70 nm in size. Using chemical reporter metabolites and a novel multifunctional fluorophore probe, here we present Lipid Expansion Microscopy (LExM), which enables the high-resolution imaging of phospholipids with a tunable expansion factor using the principles of ExM. We will present key synthetic and technological advances critical to the development of LExM as well as its application to visualize nanoscale membrane structures, phospholipid-organelle colocalization, and the spatial component of flux through specific lipid biosynthetic pathways within intact cells.

#203 - A CHEMICAL BIOLOGY APPROACH TO STUDYING INNATE IMMUNE SIGNALING

Zaro, W. B., Volk, R. F., Montaño, J. L., Warrington, S. E., and Hofmann, K. L., University of California San Francisco

Macrophages regulate how the immune system recognizes self. When a foreign/exhausted cell or pathogen is detected, macrophages engulf and destroy it in a process called phagocytosis. Anti-phagocytic signaling axes, also referred to as 'don't eat me' (DEM) signals, exist between macrophages and other cells. To evade macrophages, healthy cells express DEM signal ligands on their surface. When a DEM ligand engages a DEM receptor on a macrophage, downstream signaling blocks phagocytosis. Four DEM signal ligand-receptor pairs have been discovered, and it is hypothesized that there are others. Dysregulation of DEM signaling has been implicated in cancer, infectious disease, neurodegeneration, and atherosclerosis. In addition to DEM signal ligands, mammalian cells also present 'eat me' (EM) ligands, which engage macrophage EM receptors, and are required for phagocytosis. Despite the fundamental role these signals play in basic and disease biology, relatively little is known about the biological mechanisms and consequences of these pathways. Learning to harness and exploit DEM and EM signaling would result in a wave of new biologic discovery in human health and disease. However, highly-selective chemical probes and new specialized chemical proteomic approaches are required. To this end, over the past two years my lab has developed a more selective small-molecule modulator of EM signaling and a novel proteomics strategy to characterize differences in macrophages stimulated to promote phagocytosis. More specifically, macrophage EM signaling is regulated by Bruton's Tyrosine Kinase (BTK), and we have developed a covalent inhibitor of BTK that is 70% more selective compared to the clinically-approved inhibitor Ibrutinib. This molecule retains the Ibrutinib scaffold but is equipped with a t-butyl fumarate electrophile rather than an acrylamide. We employed chemical proteomic techniques to validate the improved selectivity of our molecule. To begin to characterize the phagocytosis proteome, we have generated a first-in-class dataset of over 6000 proteins detectable in primary human monocyte-derived macrophages that have undergone pro-phagocytic activation. We also adapted a protocol to use the methionine surrogate probe homopropargylglycine and chemical proteomic techniques to identify changes to protein synthesis in response to macrophage activation. Taken together, our new tool compounds and hypothesis-generating proteomic datasets leave us well-

poised to investigate DEM and EM signaling in human biology.

#105 - DE NOVO DESIGNED TOOLS TO MAP THE ACTIVITY/FUNCTION OF ENDOGENOUS AND ONCOGENIC RAS SIGNALING

Zhang, J., Baker, D., and Maly, D. J., University of Washington

The central signaling enzyme Ras regulates many critical functions such as differentiation, cell growth, and death. Balancing Ras signaling is critical for normal cell function as the Ras family of genes is one of the most frequently mutated in cancers, thus requiring an intricate organization of Ras and its pathway components. Ras dynamically shuttles between plasma membrane (PM), endomembranes, and surprisingly the cytosol in a membrane-independent manner from fusion RTK oncoprotein-mediated condensates, and activation of Ras at these different subcellular areas leads to diverse phenotypic outcomes. However, the mechanisms of Ras activation at these various signaling microdomains and the functional consequences of these Ras activity hotspots are unclear. Here, we will expand the molecular toolkit for Ras biology by designing tools to spatiotemporally measure endogenous Ras (sensor), profile the effectors in active Ras signaling microdomains (tracker), and perturb specific Ras activity sites especially cytosolic Ras activity by disrupting granules formed by fusion RTK oncoproteins (perturbator). The sensor and tracker is based off the de novo designed LOCKR (Latching, Orthogonal Cage/Key pRotein) switch system, which will include either split GFP or Förster Resonance Energy Transfer (FRET)-capable fluorescent protein pairs for the sensors and split TurboID for the trackers. The 2-domain perturbator protein tool contains either native or de novo chaperone binders/activators tethered to granule-targeting domains to recruit disaggregase-acting chaperones to the fusion RTK phase separated bodies. These tools will spatiotemporally map the activity/function of endogenous Ras signaling microdomains formed at different subcellular regions and by different mechanisms (dependent vs independent of membrane), aiding in our understanding of how Ras signaling specificity is encoded.

#142 - Rational Design and Characterization of a PARP16 Probe

Bejan D., Sundalam S., Jin H., Morgan R. K., Kirby I T., Siordia I R., and Cohen M. S., Oregon Health & Science University

PARP16 is an ER-resident, mono(ADP)-ribosyl transferase that has been gaining attention as a novel therapeutic target. Recent studies have revealed PARP16-dependent vulnerabilities, such as regulation of protein synthesis, that can be exploited to treat cancer. Additionally, PARP16 has been identified as an off-target of talazoparib—an approved PARP1 inhibitor—in small cell lung cancer, suggesting a potential pharmacology-based mechanism of action for talazoparib. These studies highlight the therapeutic potential for PARP16 inhibition. However, there are a lack of selective chemical tools available to validate the catalytic-dependent roles of PARP16 obtained by genetic methods (i.e., RNA interference or CRISPR). To this end, we have developed a first-in-class PARP16 probe to study the function of PARP16 in normal physiology and diseased-states.

#118 - SecreTrap - A tool to measure peptide hormone secretion at the single cell level

Bittner T., Smart K., and Schultz C., Oregon Health & Science University

Peptide hormones are essential signaling molecules that regulate various physiological functions. To date, it is still very difficult to observe peptide hormone secretion as a continuous process although its visualization is highly important for understanding the underlying molecular mechanisms and the interplay with other physiologically relevant events. Here, we present the development of a novel detection system called “SecreTrap” to monitor hormone secretion at the single cell level. The “SecreTrap” system is based on fluorescent protein-peptide fusions that are co-secreted with endogenous peptide hormones. The “SecreTrap” will trap the secreted peptide hormone construct on the cell surface, leading to accumulation of a locally defined fluorescence signal which will provide a much stronger readout than the transient release of a fluorophore. Furthermore, “SecreTrap” features an optogenetic switching tool that allow the release of cell-bound hormone-fluorescent protein fusions (HFP) by a flash of light, thereby preventing saturation of the trap. The trap and the optogenetic tool are based on the interaction of a LOV2 domain with a short Zdk1 peptide. The latter is attached to the outer membrane of the cell via a GPI anchor and traps the HFP via its additionally fused LOV2 domain to the plasma membrane. Illumination will weaken the affinity of the LOV2 domain for Zdk1 thus releasing the accumulated hormone.

#212 - Development of a Cetuximab Antibody Binding Switch Using a Novel Covalently Attached Protein L Tethered to Blocking Peptides

Brasino M., Cancer Early Detection Advanced Research Center Center, OHSU

Wagnell E., Cancer Early Detection Advanced Research Center Center, OHSU

Hamilton S., Cancer Early Detection Advanced Research Center Center, OHSU

Ranganathan S., Cancer Early Detection Advanced Research Center Center, OHSU

Gomes M., Cancer Early Detection Advanced Research Center Center, OHSU

Branchaud B., Cancer Early Detection Advanced Research Center Center, OHSU

Messmer B., Abreos Biosciences

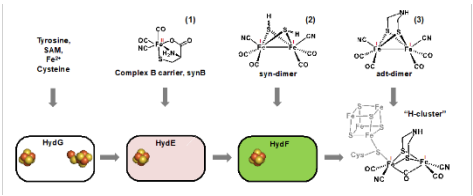
Ibsen S., Cancer Early Detection Advanced Research Center Center, OHSU

In their natural form, antibodies are always in an “on-state” where they are capable of binding to their targets. This leads to challenges with undesirable interactions in a wide range of therapeutic, analytical, and synthetic applications. Here we demonstrate a method to block the binding sites of antibodies in a predictable and reproducible way while maintaining the ability to use enzymatic activity or light to restore normal binding. This was accomplished through the design of a blocking construct that used both covalent and non-covalent interactions with a therapeutic antibody.

#122 - Enzymatic Synthesis of the Organometallic H-Cluster of [FeFe] Hydrogenase

Britt D. R., University of California, Davis

The [FeFe] hydrogenase enzymes are well suited to H2 formation, producing up to 10000 H2 molecules per second, and have therefore generated much interest for renewable energy applications. The H-cluster consists of a binuclear [2Fe]H subcluster which is linked via a bridging cysteine to a [4Fe-4S]H cluster. This [2Fe]H subcluster contains the organometallic elements of the H-cluster: the two irons each have a CO and a CN- terminal ligand and are bridged by a third CO and a unique SCH2NHCH2S azadithiolate (adt) moiety. The



H+ and H2 substrates are proposed to bind to and react at this [2Fe]H unit. In addition to the relative rarity of enzymes carrying out organometallic reactions, biosynthesis of the H-cluster poises some specific challenges. Of course, free CO and CN- molecules are toxic. In addition, the bridging adt moiety is known to be unstable in solution. The H-cluster biosynthesis is performed by a set of three "maturase" proteins, HydE, HydF, and HydG, each containing Fe-S clusters. Two of these, HydE and HydG, are members of the radical SAM superfamily of enzymes, while HydF is a GTPase. Our approach to developing a viable mechanistic proposal for H-cluster synthesis includes chemical biology techniques such as cell free synthesis, isotope sensitive spectroscopy such as electron paramagnetic resonance, and the use of synthetic clusters that can serve as functional substitutes for enzyme intermediates.

#170 - Chemoproteomic Profiling of Global Kinase Conformation by Limited Proteolysis

Brush D. S., Potter Z. E., and Maly D. J., University of Washington

Multidomain protein kinases exhibit a complex regulatory architecture consisting of auxiliary domains in addition to the catalytic kinase domain. Within the kinase domain, the architecture of the ATP-binding active site can be tightly regulated by intramolecular protein-protein interactions with auxiliary domains. This relationship bidirectional; ligands that stabilize particular active site conformations also stabilize specific global conformational changes in the kinase. This has been demonstrated in a small subset of kinases (namely amongst the SRC Family of Kinases), but whether such conformational changes occur on a kinome-wide level remain unknown. Here, a method is described that allows for high-throughput analysis of such conformational changes by Kinase-enriched Limited Proteolysis – coupled Mass Spectrometry (KLiP-MS). First, immobilized conformation-selective inhibitors are used to enrich kinases via their kinase domains. These inhibitor matrices stabilize particular active site conformations, and their corresponding global conformations. Second, inhibitors bound to the beads are subjected to limited proteolysis. Upon ligand binding, the kinase domain becomes resistant to proteolysis, while the global structural transitions that occur due to inhibitor binding render the auxiliary domains differentially susceptible to protease. The proteolysis reaction is then quenched, and kinases are digested fully. Tryptic peptides resulting from enrichment and proteolysis are monitored by LC-MS/MS, and differential rates of limited proteolysis are exhibited by statistical changes in peptide quantification throughout a time course. Using the SRC family kinases (SFKs) as a model, the KLiP-MS workflow was able to validate previous results of the global structural transitions that occur via inhibitor stabilization of active site motifs. This method is capable of elucidating structural changes in many kinases simultaneously, thus offering a high-throughput method for monitoring the conformational changes that result from ligand binding.

#138 - Structural Changes Induced in Hsp90 by Nitration Lead to a Pathological Gain-of-Function

Chatterjee T., Marean-Reardon C., Estevez A G., and Franco M C., Oregon State University

Heat Shock Protein (Hsp90) is a pro-survival molecular chaperone essential for the proper folding of a wide range of cellular proteins. It is highly abundant in most cell types and exists as a dimer that interacts with the client proteins. It is well described that the activity of Hsp90 is regulated by post-translational modifications. We showed that in pathological conditions in

which the powerful oxidant peroxynitrite is produced, Hsp90 undergoes nitration at 5 out of 24 tyrosine residues (Y) in its sequence. Further, nitration at Y33 and/or Y56 induces a pathological gain-of-function in Hsp90, leading to decreased mitochondrial activity and activation of the purinergic P2X7 receptor, which in motor neurons induces cell death. This is the first nitrated protein with a pathological function described to date. Nitration of Hsp90 is of particular relevance in amyotrophic lateral sclerosis (ALS), a motor neuron degenerative condition with an elusive pathogenesis and scant treatment options. We discovered that Hsp90 is endogenously nitrated in motor neurons in the spinal cord of ALS patients, the cell type that is compromised in this disease process. Establishing the structural changes induced in Hsp90 by nitration that lead to the pathological toxic function is crucial for the development of novel therapeutic strategies for ALS treatment. Here, using a combination of biophysical approaches we show that nitration of Hsp90 at Y33 and Y56, the residues relevant to the toxic function, had a profound impact on Hsp90 structure. To dissect the contribution of nitration at Y33 and/or Y56 on Hsp90 structural changes, we performed analytical ultracentrifugation sedimentation velocity experiments using recombinant Hsp90, peroxynitrite-treated Hsp90 (fully oxidized protein), and site-specific nitrated Hsp90 produced by genetic code expansion, carrying nitrotyrosine at either position 33 or 56, or simultaneously at both positions as the sole modification in the protein. Following peroxynitrite treatment, Hsp90 dimer was destabilized, showing a significant increase in the number of monomers, and the formation of oligomeric species. These results were confirmed by negative stain cryo-electron microscopy, and size exclusion chromatography followed by multiangle light scattering. Site-specific nitration at Y33 or Y56 was enough to destabilize the dimer conformation, while simultaneous nitration at Y33 and Y56 most closely resembled the structural changes observed after peroxynitrite-treatment. In contrast, replacement of Y33 and Y56 by nitration-resistant phenylalanine decreased dimer destabilization and prevented oligomer formation after peroxynitrite treatment. Together, these results suggest that selective nitration at Y33 and Y56 is enough to significantly affect the global structure of Hsp90, and that these changes may be responsible for nitrated Hsp90 toxic gain-of-function. Thus, targeting these structural changes may lead to the development of drugs that selectively inhibit nitrated Hsp90 pathological activity in ALS, without affecting the function of the unmodified chaperone in normal tissues. Supported by NIH/NINDS R01NS102479 (to MCF).

#188 - IDENTIFICATION OF THE TARGETS OF BETA-LACTAM ANTIBIOTICS IN MYCOBACTERIUM TUBERCULOSIS

Dearing H., Oregon Health & Science University

Levine S., Oregon Health & Science University and UC Irvine

Beatty, K., Oregon Health & Science University

Tuberculosis (TB) has infected humans for thousands of years and killed 1.5 million people in 2020. Infections caused by drug-susceptible *Mycobacterium tuberculosis* (*Mtb*) are curable, although treatment usually requires at least 6 months of therapy with multiple antibiotics. Unfortunately, TB has become increasingly difficult to treat due to the spread of drug resistant strains. Such strains are found globally, and the cure rate for these patients is only about 54%. New drugs are urgently needed to treat drug-resistant strains, but drug development is a slow and costly process. However, there is some evidence that clinically-approved β-lactam antibiotics could be repurposed to treat infections with drug susceptible or drug resistant *Mtb*. The addition of β-lactam antibiotics to TB treatment regimens could be a game changer because these drugs are widely available, well-tolerated, and cheap. In mycobacteria, β-lactam antibiotics target enzymes in the cell wall, including the penicillin-binding proteins (PBPs) and L,D-transpeptidases (LDTs). These two classes of enzymes maintain the structure and rigidity of the cell wall, making them essential for *Mtb* survival. We have recently described a small set of activity-based probes that can detect PBP and LDT activity, including probes derived from a monobactam (aztreonam-Cy5), a cephalosporin (cephalexin-Cy5), and a carbapenem (meropenem-Cy5). We have also developed a meropenem-biotin probe for affinity enrichment of enzyme targets. We will describe our progress using these probes to study the activity, drug susceptibility, and

regulation of PBPs and LDTs in both dormant (non-replicating) and active *Mtb*.

#166 - FUNCTIONAL VALIDATION OF KATP CHANNEL PHARMACOLOGICAL CHAPERONE CANDIDATES FROM CRYOEM STRUCTURE-BASED VIRTUAL SCREENS

ElSheikh A., Zhongying Y. (retired), Driggers C. M., and Shyng SL. Oregon Health & Science University

In pancreatic β-cells, ATP-sensitive K+ channels (K_{ATP} channels) couple glucose metabolism to insulin secretion by adjusting plasma membrane excitability. Genetic mutations that disrupt pancreatic K_{ATP} channel biogenesis, folding, assembly, trafficking, and plasma membrane anchoring, collectively referred to as trafficking defects, lead to a severe form of the metabolic disorder congenital hyperinsulinism (CHI) due to impaired control of insulin secretion [(1)]. Pharmaco-chaperones (PCs) are target-specific, small molecules that bind to their target proteins to facilitate biogenesis and/or prevent/correct misfolding and in some cases restore full or partial function of misfolded protein to reverse disease phenotypes. Therefore, the use of PCs holds great promise as a novel therapeutic avenue for the treatment of protein folding disorders for which there are no effective therapies. Our group has shown previously that sulfonylurea drugs, small molecules that are used as K_{ATP} channel inhibitors for the treatment of type 2 diabetes, and carbamazepine, a drug used as anticonvulsant, are efficient PCs that correct trafficking defects in a subset of sulfonylurea receptor 1 (SUR1) mutants, a subunit of the pancreatic K_{ATP} channel [(2)]. However, the use of these drugs for correcting misfolded K_{ATP} channel defects in CHI is hampered by high affinity and irreversible binding of these drugs to pancreatic K_{ATP}, which prevents function of rescued channels, or by their undesired side effects on different K_{ATP} channel subtypes in other organs [(3)]. Therefore, there is a need to identify additional compounds that can correct K_{ATP} channel trafficking defects without compromising function and with greater K_{ATP} subtype-specificity. To this end, in a collaborative effort with Atomwise, a company that specializes in structure-based virtual screening, we recently conducted virtual screening using cryoEM structure of SUR1 bound to a sufonylurea drug and identified three compounds that have promising PC effects on SUR1-F27S, a trafficking mutation known to be corrected by sulfonylurea drugs. Here, using both electrophysiological and biochemical methods, we are aiming to test the PC effects of these drugs over different subsets of already known K_{ATP} channel mutations involved in CHI, and to assess the function and activity of the surface rescued K_{ATP} channels.

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#148 - SPONTANEOUS PEPTIDE MACROCYCLIZATION THROUGH P-CYANOACETYLENE-PHENYLALANINE, A ROBUST AND RELIABLE TECHNOLOGY

Estheban O., Chaloux B., and Hartman M., Virginia Commonwealth University

Peptides as therapeutics have been growing in interest in the last two decades due to their ability to interrupt protein-protein interactions (PPI) associated with a wide variety of critical diseases such as cancer. Protein surfaces are typically too large and featureless for small molecules to bind effectively. On the other hand, peptides already exhibit extended surface areas that are composed of amino acids. More than 60 peptide drugs have been approved by the FDA, two-thirds of those being peptide macrocycles (PMC). However, when it comes to intracellular environments, peptides are also the target of proteases which lead subsequent degradation and excretion. Here is when peptide cyclization enters as an alternative to overcome such issues, conferring rigidity and inaccessibility to the protease's active site; furthermore, cyclization also presets a conformational shape that decreases binding entropy resulting in tighter interactions with their targets improving binding affinity. Those advantages have motivated researchers to find new ways to make cyclic peptides; however, most cyclization chemistries are incompatible with translation or display technologies. To the best of our knowledge, only two other spontaneous and irreversible

cyclization chemistries have been described that are compatible with translation, Suga's α-chloroacetamide chemistry, and Fasan's MOrPh-PhD. Here we describe a new peptide cyclization technology that incorporates the non-canonical amino acid *p*-cyanoacetylene-phenylalanine (pCAF). This amino acid is incorporated in place of phenylalanine and is charged onto $tRNA^{Phe}$ by the *E. coli* PheRS (A294G). pCAF reacts through a Michael addition mechanism with cysteine thiols and can make a PMCs with various ring sizes. The cyclized products are stable to reducing agents such as DTT or BME. The high cyclization efficiency and reactivity also allow double cyclization leading to highly constrained bicyclic peptides. Although this technique is applied to in-vitro translation systems and does require the exchange of F for pCAF, our lab is exploring other alternatives to assign this novel amino acid to additional codons through amber suppression to expand such application to site-directed modifications for protein conjugation.

#178 - EXPLORING THE RELATIONSHIP BETWEEN LC8 AND VIRAL PHOSPHOPROTEINS

Fujimura G., and Barbar E., Oregon State University

All mammalian cells contain a protein 'hub' known as LC8, which is essential for multiple protein complexes involved in many parts of the cell. LC8 actively binds to its partner proteins, most of which are highly disordered, to promote self-association and higher order organization. We are investigating the binding of LC8 to viral phosphoproteins, namely HPIV-3 and Rabies. We have specific interest in the Rabies Phosphoprotein (RavP) which is involved in viral genomic replication. It is a highly disordered large protein complex, which is already known to bind to LC8. There are several human proteins that may also bind to RavP at the same location LC8 does--disrupting RavP's structure and inhibiting polymerase activity. This research has potential for investigating a deeper understanding of virus-LC8 interactions that span not only in Rabies but in all viruses that may bind to LC8.

#194 - SUBCELLULAR DELIVERY OF HYDROGEN SULFIDE VIA SMALL MOLECULE DONORS

Gilbert A. K., and Pluth M. D., University of Oregon

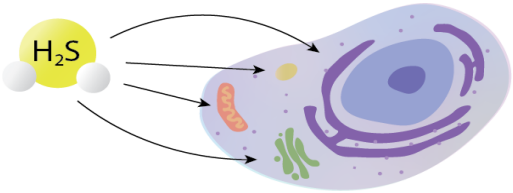
Hydrogen sulfide (H_2S) is an important biological molecule that is endogenously produced. H_2S is involved in a variety of physiological processes, such as providing antioxidant and anti-inflammatory effects, promoting angiogenesis, and promoting bone formation. Although H_2S is an ideal candidate as a potential therapeutic molecule, controlling the release and localization of H_2S delivery is a challenge because of its reactivity and gaseous state. To address these challenges, small molecule H_2S donors have been developed to achieve a slow sustained release of H_2S that contrasts the large bolus release of H_2S as a gas or inorganic sulfide salt. Furthermore, these small molecule donors can be activated by specific biological stimuli or incorporate targeting groups to localize H_2S release to areas of therapeutic need. Although mitochondrially-targeted H_2S donors, such as AP39, have been reported previously and exhibit significantly higher potency than non-targeted donors, the expansion of targeted H_2S delivery to other subcellular organelles remains absent. To fill this key unmet need in the field, we have prepared a library of esterase-activated thiocarbamate based H_2S donors appended with targeting groups that specifically localize in the endoplasmic reticulum, the Golgi apparatus, the lysosome, and the mitochondria. We confirmed H_2S production from each of these donors using an H_2S selective electrode and confirmed localization of H_2S delivery using fluorescent cell imaging. Ongoing research includes the application of these donors to cells induced with specific organelle stress to investigate subcellular roles of H_2S.

#206 - PT(II)-BASED COMPOUNDS TO ELUCIDATE MOLECULAR MECHANISMS INVOLVED IN THE NUCLEOLAR STRESS RESPONSE

Guerrero A S., McDevitt C. E., Smith H. M., and DeRose V. J., University of Oregon

Platinum(II)-based chemotherapeutic compounds are one of the most widely used in clinical settings and include the three FDA-approved compounds: cisplatin, carboplatin,

and oxaliplatin. Until recently, it was believed that all of Pt(II)-based compounds induced apoptosis through the DNA damage response (DDR), but in 2017 it was discovered that oxaliplatin instead induces cell death through a unique nucleolar stress response. The mechanisms by which Pt(II) compounds interact with the nucleolus to cause this specific inhibition of ribosome biogenesis and subsequent cell death are not understood. Through limited structure-function analyses we previously determined that the diaminocyclohexane (DACH) ring of oxaliplatin is important for nucleolar stress induction. Our current efforts involve modification of the DACH ring 4- position to explore thresholds for steric bulk and orientation that still support nucleolar stress. These studies inform plans to install a clickable azide reporter on the DACH ring, allowing post-treatment Cu-catalyzed azide-alkyne cycloaddition (CuAAC) of reporter groups. Downstream detection and identification of the nucleolar targets of these Pt(II) compounds will be compared to results obtained previously in the DeRose laboratory with clickable Pt(II) compounds that mimic cisplatin, which does not induce nucleolar stress. These studies will lead to an understanding of how Pt anticancer compounds interact with components of the nucleolus, and how the ligands of Pt compounds might direct them to cause nucleolar-based cell death.



#130 - Efficient Visible/NIR Light-driven Uncaging of Hydroxylated Thiazole Orange-based Caged Compounds in Aqueous Solution

Hashimoto R., Osaka University

Minoshima M., Osaka University

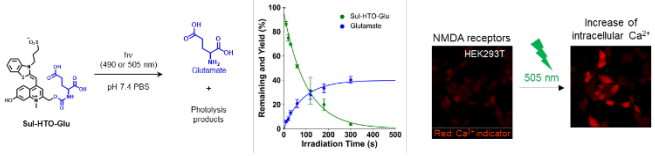
Kikuchi K., Osaka University and Immunology Frontier Research Center

Caged compounds are powerful tools for non-invasive spatial and temporal control of bioactivity and are produced by incorporating photoremovable protective groups (PPGs) into the structure of bioactive compounds. Illumination results in the release of the biomolecules and a subsequent concentration jump, which allows binding to cellular receptors and switching on or off of the targeted process. Unfortunately, the most PPGs require high-energy UV-light illumination, which leads to cellular damage or death at a low tissue penetration depth. In the biological setting, longer-wavelength light uncaging is desirable. Only a limited number of visible-light-sensitive PPGs (>450 nm) based on ruthenium complexes, boron dipyrromethene (BODIPY) derivatives, or cyanine dyes have recently been reported.[1] However, the visible-light-sensitive PPGs reported so far have displayed high hydrophobicity and low photolytic efficiencies in aqueous solution ($\varepsilon\Phi < 50$), which can obstruct the control of bioactivity with high spatial and temporal precision. As an alternative to long-wavelength light uncaging methods, two-photon excitation (2PE) with near-infrared (NIR) light provides deeper tissue penetration, reduced phototoxicity, and high 3D resolution. Although some UV/blue-light-sensitive caged compounds can be activated by two-photon excitation (~740–800 nm), fewer two-photon excitation-sensitive chromophores are available to long-wavelength light (~900 nm) for biological applications.[2] Therefore, the development of long-wavelength light-sensitive PPGs with high cleavage efficiency in aqueous solution is still a significant challenge.

In this study, we present a new molecular framework for one-photon visible (490–505 nm) or two-photon (940 nm) excitation-responsive PPGs with high cleavage efficiencies in aqueous solution. We rationally designed and synthesized hydroxylated thiazole orange dye-based PPGs (HTO), which possess efficient photodegrading structures (7-hydroxyquinoline/quinolinium moieties[3],[4]) in a visible-light-absorbing chromophore. Importantly, the uncaging efficiencies ($\varepsilon\Phi \approx$ 110~370) of HTO-caged compounds were superior to those reported for visible-light-responsive PPGs in the aqueous condition. In addition, the 2PE photolysis reaction of a glutamate-conjugated HTO-caged compound (Sul-HTO-Glu) was achieved using an NIR laser (940 nm). These results demonstrate that HTO has the potential to immediately release the biomolecule and activate target biological functions via single-photon visible or two-photon NIR light illumination. Finally, we demonstrated optical control of N-methyl-D-aspartic acid (NMDA) receptors in Xenopus

oocytes and mammalian HEK293T cells with Sul-HTO-Glu. The illumination of 505-nm light on Sul-HTO-Glu successfully released glutamate under biological conditions and activated NMDA receptors with controlled timing.

These results demonstrate that Sul-HTO-Glu has efficient visible-light uncaging properties in aqueous solution and spatiotemporal activation of NMDA receptors in cells. The excellent optical properties of HTO-caged compounds can allow applications such as controlling multiple biological functions or exploring cellular function in cultured cells, as well as in living tissues or animals.



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#182 - Estimating Opioid Receptor Copy Number with Covalent, Fluorescent Labelling

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Improvements in cellular biology are crucial to further unveiling the intracellular activity of GPCRs, a highly desirable target for drug discovery. Opioid receptors are a particularly compelling area of GPCR research because they exhibit ligand bias, that is, the variable intracellular effects by the same opioid receptor under exposure to different ligands. Further research in ligand bias of opioid receptors will provide useful information underpinning therapeutic strides in opioid analgesia therapy. Modelling of receptor activity can involve transfection of human cells with a tagged receptor of interest. However, the plasmids in which these receptors are expressed result in a constitutively-high expression levels, unlike endogenous levels. Above-normal GPCR expression has sparked concern that it may cause several undesirable outcomes, including potentially aberrant oligomerization of receptors and atypical interactions between receptors and downstream effectors. Promoter modification could provide a means to 'fine-tune' GPCR levels to near-endogenous levels, if only the number of receptors could be known or compared. The development of naltrexamine-acylimdiazole-AlexaFluor488 (NAI-A488), a ligand-directed tag that covalently attaches a fluorochrome to opioid receptors, could provide the solution to this conundrum. Namely, this reactive opioid tag will provide a raw fluorescence per cell via flow cytometry that may be compared to the fluorescence of beads with variable known number of conjugated molecules of fluorochrome. Therefore, we sought to identify saturation conditions of NAI-A488 on various mu opioid receptor expressing cell lines, including endogenous ones such as SY5Y, and stably transfected HEK293 lines using UBC and CMV promoters to identify receptor number.

#134 - THRONCAT: Efficient Metabolic Labeling of Newly Synthesized Proteins Using a Bioorthogonal Threonine Analog

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The cellular proteome consists of a complex and dynamic protein pool that is tightly regulated by protein synthesis and degradation. Changes in protein synthesis can occur rapidly in response to internal and external stimuli and capturing these early responses provides invaluable insight into cellular physiology. Measuring this nascent proteome requires a method to distinguish between newly synthesized proteins (NSPs) and the pre-existing proteome. To this end, metabolic protein labeling using bioorthogonal methionine analogs (BONCAT) has been used extensively to tag and enrich NSPs. Methionine analogs, however, are poorly incorporated and efficient BONCAT relies on the absence of methionine in the cell, practically limiting the use of BONCAT to methionine-free culture media and methionine-auxotrophic strains of bacteria. Here, we present a novel metabolic labeling method based on bioorthogonal threonine analogs (THRONCAT) that are efficiently incorporated into NSPs in complete growth medium and in wild-type cells in vitro and in vivo. Our data show that bioorthogonal threonine analogs allow for visualization of

NSPs on a minute time scale as well as efficient enrichment of NSPs for proteomic analysis. Because of its ease, we believe that THRONCAT find widespread applications to study protein regulation and dynamics in health and disease in more detail.

#186 - Tuning Tetrazine Amino Acid Size, Reactivity and Stability of For Efficient Protein Labeling

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Site-specific fast protein labeling using genetically encoded tetrazine (Tet) amino acid requires synthetic access to Tet amino acids, genetic engineering, and then rigorous characterization of Tet amino acids reactivity and stability balance of under biological condition. To overcome the limitations and access a various substituted 1,2,4,5-tetrazine amino acids (Tet-ncAA) we have synthesized a series of Tet-v2.0, Tet-v3.0 and Tet-v4.0 ncAAs. Each set of tetrazine amino acids decorated with different aliphatic and aromatic substituents to tune the reactivity and stability. For intracellular protein labeling, rapid quantitative bioconjugation reaction are in high demand. Maintaining high biorthogonal reaction rates with undetectable side-reactions using small tetrazine-ncAA that don't compromise protein structure is challenging. Here, we investigated the synthetic accessibility and a wide range of reactivity and stability of various Tet-ncAAs inside protein. The Tet-v4.0 amino acids inside the protein offers the combination of desired features including small, ultra-fast kinetics (>106 M-1s-1), quantitative labeling and stable linkage with sTCO reagents. We demonstrate the Tet-v4.0 can be incorporated at different single and double user-defined sites of maltose binding protein (MBP) and generate a shortest linkage sTCO-Nitroxyl spin labels to attach a EPR probe as close as possible to the protein backbone.

#196 - Probing tertiary structure and RNA-RNA interactions in the SARS-CoV-2 genome via cisplatin crosslinking

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To improve understanding of the structure and function of the SARS-CoV2 virus, and to inform the development of therapeutics against COVID19, accurate 3D models of SARS-CoV2 RNA structures are essential. SARS-CoV2 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-CoV2 RNA structures through the identification of tertiary contact points by means of chemical crosslinking. The DeRose lab has previously shown that cisdiamminedichloroplatinum(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. Recent work has focused on characterizing interactions involving the untranslated regions of the viral genome, which have been found to serve essential functions in transcription. Identified crosslinks will refine current models of SARS-CoV2 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.

#200 - TRIFUNCTIONAL PIP3 AS A TOOL FOR DISCOVERING NEW PIP3 BINDERS AND TRANSPORTERS

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Building on our extended experience in the synthesis of functionalized phosphoinositides, we recently synthesized a multifunctional version of phosphatidylinositol 3,4,5-trisphosphate (TF-PIP_3/AM) featuring a coumarin caging group, a cross-linkable diazirine group, an alkyne group for click chemistry and bioactivatable acetoxymethyl esters for passive cell entry.(Mueller et al., Angew. Chem. 2021) The caging group keeps the molecule inert until it is photolyzed by a flash of 400 nm light. Photo-crosslinking is orthogonally induced by 350 nm UV light. TF-PIP_3/AM loaded into intact cells within a few minutes where is preferentially localized in ER membranes. Subsequently, we exposed cells to 400 nm light for 2 min to release active PIP_3. We then illuminated at 350 nm and triggered the cross-linking of the compound to its interactors. Cells were lysed and the lipid-protein conjugates were tagged to azide functionalized beads in the presence of copper(I) ions. Enriched proteins were digested and analyzed by mass spectrometry. We compared the detected proteins to a list of proteins known or predicted to have a lipid binding domain and selected six overlapping proteins of which three were chosen for further testing. We found that knock-down of two of them (MPP6 and ATP11A) by siRNA reduced trafficking of our PIP_3 derivatives from endomembranes to the plasma membrane. Incubation of MPP6-GST purified protein with membrane decorated with immobilized phospholipids showed specific and potent binding to PS and phosphoinositides containing a phosphate group at either position 3 or 5. Construction of a GFP tagged MPP6 construct showed that the protein is preferentially located at internal membranes and that the PDZ and SH3 domains of MPP6 are essential for lipid binding. The surprising transport of PIP_3 between cell membranes might explain why PH domains that recognize PIP_3 will only find their ligand at the plasma membrane.

#201 - NON-LIGAND INDUCED INTERNALIZATION OF EGFR

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The epidermal growth factor receptor (EGFR) is activated by its ligand (EGF) at the plasma membrane. Activated EGFR dimerizes, the dimers cross-phosphorylate (tyrosine phosphorylation) and become ubiquitinated. About 2 to 4 min later, EGFR is internalized into clathrin coated pits. If ubiquitination is present at its C-terminus, EGFR will be sorted for lysosomal degradation. We previously found that elevated levels of phosphatidylinositol 3,4,5-trisphosphate (PIP3) also lead to internalization but without a need for dimerization and in the absence of Tyr phosphorylation and ubiquitination. These receptors are exclusively recycled back to the plasma membrane. It is currently unclear what the mechanism and the recognition pattern for non-ligand induced internalization is. We hypothesized that Ser/Thr phosphorylation is providing the internalization trigger. We therefore opted for an unbiased proximity labeling approach combined with proteomics. We tagged the C-terminal tail of EGFR with the APEX2 engineered peroxidase and treated cells with a membrane-permeant derivative of PIP3, in the presence of biotin-phenol and hydrogen-peroxide. This resulted in the biotinylation of proteins that interact with EGFR in the presence of elevated PIP3 levels. Proteomic analysis revealed an enrichment of PKA catalytic subunits alpha and beta in cells treated with PIP3 versus control. To confirm the relevance of this result, we incubated EGFR-GFP transfected cells with PKA inhibitors (H89 or PKI) and observed a significantly reduced number of EGFR positive vesicle compared to non-treated cells. Further, the start of internalization was delayed from 2-4 min post-treatment to 5-8min post-treatment. Even though we observed a decrease in internalization, we didn't see full abolishment. For this reason, we tested several other serine/threonine kinase inhibitors and found that inhibition of p38k fully abolished EGFR internalization after elevating PIP3 levels. Based on the involvement of

serine/threonine kinases in the process, we wondered which of the many C-terminal Ser/Thr residues of EGFR were phosphorylated. Mutation of serine and threonine residues to alanine in the regulatory C-tail (residues in region aa1028-1041) showed a decrease in EGFR internalization after elevating PIP3 levels. More specifically, mutation of p38k phosphorylated residues S1039T1041 to alanine also showed a decrease in internalization, but not full abolishment as in the presence of a p38k inhibitor. However, when we treated cells expressing the S1039T1041A mutant tagged with GFP (p38k phosphorylated residues mutated) with PKA inhibitors, internalization was halted. These results suggest that in the presence of elevated PIP3 levels, p38k and PKA are activated downstream of PIP3 which leads to the Ser/Thr phosphorylation of EGFR, non-ligand induced internalization and recycling. Ser/Thr phosphorylation of EGFR seems to provide the signal for cargo recognition.

#184 - MOLECULAR LEVEL CONSEQUENCES OF MRNA URIDINE MODIFICATIONS ON TRANSLATION

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Cells face the daunting challenge of synthesizing the correct number of proteins at the right time with high fidelity. Messenger RNAs (mRNAs) serve as the blueprints for protein synthesis by the ribosome, and the post-transcriptional modification of mRNA presents one avenue for cells to regulate protein production. The recent discovery of mRNA chemical modifications has generated tremendous excitement because these modifications have the potential to regulate mRNA maturation, translation and stability to control protein expression levels. Here, we systematically investigate the molecular level consequences of translating mRNAs possessing modified uridine nucleosides. We selected pseudouridine (Ψ), 5-methyluridine (m^5U) and N1-methylpseudouridine ($m^1\Psi$) for study because they are present in either naturally occurring mRNAs (Ψ and m^5U) or serve as key components of emerging mRNA-based vaccine and therapeutics ($m^1\Psi$). We used a fully bacterial reconstituted translation system to directly assess how protein synthesis is impacted by these modifications. Our work reveals that replacing a uridine with a modified base in an mRNA codon can reduce the rate of amino acid incorporation, and promote the synthesis of multiple peptide products from a single mRNA sequence. In our system, the impact of individual modifications on translation is highly dependent on the surrounding mRNA sequence context. These *in vitro* findings are corroborated by studies in yeast and human embryonic kidney cells demonstrating that the uridine modifications can slow translation and increase the occurrence of amino acid misincorporation by the ribosome. To investigate the chemical logic for our biochemical and cellular observations we computationally modeled a cognate and near cognate tRNAs bound to both unmodified and modified mRNA phenylalanine codons. These data suggest that enthalpically driven changes in mRNA:tRNA interactions largely account for the context specificity of the miscoding events that we observe. Our findings provide support for the hypothesis that chemical modifications in mRNA can potentially provide a mechanism for cells to quickly and directly modulate the speed and accuracy of protein production.

#114 - PRMT5 AS A NOVEL DRUGGABLE VULNERABILITY FOR EWSR1-ATF1-DRIVEN CLEAR CELL SARCOMA

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Clear cell sarcoma of soft tissue (CCSST) is a rare and aggressive soft tissue sarcoma. The 5-year survival rate is only 20% for metastatic cases of CCSST. CCSST is insensitive to existing chemotherapies or radiotherapy. New target therapies will have tremendous impact on CCSST patients. The hallmark of CCSST is a chromosome translocation, which generates an oncogene EWS-ATF1/CREB, where Ewing's sarcoma gene EWSR1 (EWS RNA-bind protein 1) is fused with activating transcription factor 1 (ATF1) or cAMP response element binding

protein (CREB). EWS-ATF1/CREB is a constitutively active form of ATF1/CREB and is independent of extracellular cues to drive ATF1/CREB-dependent gene transcription. We recently discovered that protein arginine methyltransferase 5 (PRMT5) is involved in EWS-ATF1-mediated gene transcription. Genetic silencing of *PRMT5* in CCSST cells results in severely inhibited cell growth. We further identified a PRMT5 inhibitor **JNJ-64619178** to potently and efficaciously inhibit CCSST cell growth *in vitro* and *in vivo*. This becomes clinically very relevant as **JNJ-64619178** finished phase I clinical trial (NCT03573310) in advanced solid tumors and non-Hodgkin lymphoma patients and it demonstrated manageable toxicity. Our results presented here provide a strong mechanistic basis for further clinical evaluation of **JNJ-64619178** to treat CCSST patients.

#128 - DISCOVERY OF NON-CANONICAL PROTEIN TARGETS OF FENTANYL ACROSS TISSUES FROM ANIMAL MODELS AND HUMANS USING PHOTOAFFINITY FENTANYL PROBES

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Synthetic opioids such as fentanyl and related analogs have been widely used for pain management. However, their negative side effects, including constipation, respiratory depression, and high potential for addiction underscore the need for a deeper understanding of fentanyl's interactions with various cellular receptors throughout the human body. Fentanyl analogs bind and activate opioid receptors in the central and peripheral nervous systems, triggering a variety of downstream signaling pathways. Increasingly, fentanyl has been shown to interact with non-opioid receptors, and elucidation of these non-canonical fentanyl-protein interactions may provide insight into the mechanisms contributing to fentanyl's adverse effects and inform future drug designs or medical countermeasures. To identify proteins in mammalian tissues that may interact with fentanyl, we designed and synthesized three fentanyl analog probes featuring a diazirine photoaffinity group and alkyne handle for click chemistry at different positions. Molecular docking simulations confirmed that the physicochemical properties and mu opioid receptor binding scores of these probes were similar to fentanyl. Application of these probes to chemoproteomic profiling of cells and tissue homogenates is underway to characterize proteins that may interact with fentanyl in different organs and animal models.

#140 - NITRATION OF HSP90 AFFECTS ITS SPATIAL DISTRIBUTION AND PROMOTES SCHWANNOMA CELL PROLIFERATION

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Neurofibromatosis Type 2 is a genetic disorder characterized by the development of schwannomas throughout the nervous system caused by loss of merlin tumor suppressor activity. Solid tumors develop in an oxidative environment due to production of oxidants such as peroxynitrite by tumor and infiltrating immune cells. We discovered peroxynitrite production and subsequent protein tyrosine (Y) nitration support schwannoma cell survival/proliferation. Further, Y nitration induces a metabolic reprogramming characterized by decreased oxidative phosphorylation activity, and increased glycolysis and glutaminolysis. Here, we identified the first nitrated protein supporting schwannoma cell proliferation, the molecular chaperone heat shock protein 90 (Hsp90). Out of five Y residues prone to nitration in Hsp90, we showed nitration at Y33 downregulates mitochondrial metabolism, while nitration at Y56 activates the ATP-gated receptor P2X7, which increases glycolysis in tumor cells. We found schwannomas contain endogenous levels of Hsp90 nitrated at either position. Therefore, we hypothesized that nitrated Hsp90 (Hsp90NY) promotes schwannoma cell proliferation by regulating their metabolism. To investigate the role of Hsp90NY in proliferation, we increased the intracellular concentration of Hsp90NY in schwannoma cells by delivering Hsp90NY intracellularly at concentrations like those endogenously present in these cells (~10% of cellular Hsp90, calculated by quantitative dot blot). Doubling

the intracellular concentration of Hsp90NY but not wild-type Hsp90 significantly increased cell proliferation 24 h post-delivery. To establish the metabolic role of Hsp90NY, we delivered wild-type or Hsp90NY into wild-type Schwann cells (WT-SC) and studied the metabolic changes by extracellular flux analysis 24 h post-delivery. Hsp90NY decreased all parameters of mitochondrial activity, with no effect on the glycolytic rate. To test if Y33 and/or Y56 were responsible for the tumorigenic activity of Hsp90NY, we intracellularly delivered to WT-SC either wild-type Hsp90, or different site-specific nitrated Hsp90 proteins produced by genetic code expansion: Hsp90 nitrated at Y33 (Hsp90NY33), at Y56 (Hsp90NY56), or at Y33 and Y56 simultaneously (Hsp90NY33+56). All three forms of Hsp90NY significantly increased WT-SC proliferation at 24 and 48 h post-delivery, suggesting Y33 and Y56 induce schwannoma cell proliferation independently. Using a three-dimensional schwannoma cell culture model (tumoroids), we discovered Hsp90NY33 colocalized with mitochondria and remained in the tumoroid periphery, while Hsp90NY56 was also found in nuclei with a homogenous distribution throughout the tumoroid. Collectively, our results show that Hsp90, when nitrated at Y33 or Y56, gains a tumorigenic activity that supports schwannoma cell proliferation. In addition, residue-specific nitration results in distinct localization, and potentially function, of the protein. This is the first nitrated protein shown to support tumor cell proliferation, and a potential tumor-directed target for therapeutic development.

#202 - Improved Electrophile Design for Exquisite Covalent Molecule Selectivity

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Covalent inhibitors continue to show therapeutic promise. However, off-target reactivity challenges the field. Extensive efforts have been exerted to solve this issue by varying the reactivity attributes of electrophilic warheads, with features such as reversibility or metabolic vulnerability. Here we report the development of a new approach to increase the selectivity of covalent probes and small molecule inhibitors that is independent of warhead reactivity features and can be used in concert with already-existing methods. Using the Bruton's Tyrosine Kinase (BTK) inhibitor Ibrutinib scaffold for our proof-of-concept, we reasoned that increasing the steric bulk of fumarate-based electrophiles on Ibrutinib should improve selectivity via the steric exclusion of off-targets but ideally retain rates of cysteine reactivity comparable to that of an acrylamide. Using chemical proteomic techniques, we demonstrate that elaboration of the electrophile to a tert-Butyl (t-Bu) fumarate ester significantly decreases time-dependent off-target reactivity and abolishes time-independent off-target reactivity but retains BTK target engagement. While an alkyne-bearing probe analog of Ibrutinib has 247 protein targets, our t-Bu fumarate Ibrutinib probe analog has only 7 protein targets. Of these 7 targets, BTK is the only time-independent target. This increase in selectivity is also conferred to the t-Bu inhibitor itself, reducing off-targets by 70%. By shotgun proteomics, we investigated the consequences of treatment with Ibrutinib and our t-Bu analog and discovered that only 8 proteins are downregulated in response to treatment with the t-Bu analog compared to 107 with Ibrutinib. Of these 8 proteins, 7 are also downregulated by Ibrutinib and a majority of these targets are associated with BTK biology. Taken together, these findings reveal a previously-unappreciated opportunity to increase cysteine-reactive covalent inhibitor selectivity through electrophilic structure optimization.

#162 - Chasing CIITA: Preliminary Structure Investigation with CryoEM

Nash G., Cao J., Paidimuddala B., and Zhang L., Oregon Health & Science University

The Class II Transactivator, CIITA, is a valuable and challenging target for structural biology research. CIITA is a member of the NLR protein family and the master regulator of MHC class II expression. The Major Histocompatibility Complex (MHC) is responsible for presenting cell-surface antigens for recognition by T cells. MHC class II plays a vital role in adaptive immunity as it presents exogenous antigens, in contrast to the endogenous antigens presented by class I. Immunodeficiency such as bare lymphocyte syndrome (BLS) can be caused by the absence of proper CIITA activity, and MHC class II expression is commonly absent in tumors.

Determining the structure of CIITA holds significant potential for development of novel treatment for these conditions.
This project seeks to investigate the structure of CIITA via cryoEM. Sf9 insect cells are used to express recombinant CIITA. Initial efforts centered on increasing CIITA expression to a level that would result in a concentration suitable for grid preparation following size exclusion chromatography (SEC). This was accomplished in two phases. A series of small-scale purifications were performed with the cultures harvested at various times post-infection to determine the optimal harvest time. A set of baculovirus stocks were then generated with various CIITA deletion mutants to increase the stability of the protein, and a second series of small-scale purifications were performed for evaluation. With the calibrated harvest time and using the best-expressing mutant, expression was significantly increased. Subsequent purifications were able to advance to the grid preparation stage.
The current focus is on overcoming the challenges posed by the aggregation-prone nature of CIITA and by the presence of a contaminant protein that co-elutes on the SEC column. Improvements have been observed during grid screening as adjustments to the grid buffer conditions have been implemented. This has allowed preliminary data collection, but the resulting 2D class images have been identified as the contaminant protein.
Future efforts will entail optimizing the purification strategy to remove the contaminant protein and additional screening for buffer conditions to reduce CIITA aggregation. Success may allow for the development of new treatments for some cancers and conditions of immunodeficiency related to CIITA & MHC class II expression. Furthermore, lessons learned from the process may assist in efforts to solve structures for similar proteins including other NLR family members.

#108 - Services Available at the OHSU Medicinal Chemistry Core

Dr. Tapasree Banerji T., Devereaux J., Halls V., and Nilsen A., Oregon Health & Science University

The poster will show examples of services that are available at the OHSU Medicinal Chemistry Core including those related to chemical biology, medicinal chemistry, custom organic and peptide synthesis, and fee-for-use instrumentation.

#164 - Genome-wide CRISPRi Screen Reveals RME8/DNAJC13 as a Novel Mediator of Delta-Opioid Receptor Regulation

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Over half of drug-related deaths in 2020 were opioid or synthetic-opioid related. These opioids act upon the opioid receptor family, producing a downstream pain-relief response with historically addictive capacities. To better understand the mechanisms underlying opioid receptor regulation, we conducted a genome-wide CRISPRi screen using the Delta-Opioid Receptor as our target. Using a fluorescent reporter sensing lysosomal degradation, we were able to establish key genes that affect Delta-Opioid Receptor transport. Our screen revealed the protein RME8/DNAJC13 as a mediator of degradation for the Delta-Opioid Receptor. Further investigation of RME8 revealed it's localization at the base of retromer-positive tubules and an association with Delta-Opioid Receptor trafficking endosomes. After siRNA-mediated knockdown of RME8 we observed a delay of Delta-Opioid receptor transport to the lysosome, as well as a change in endosomal morphology resulting in larger and more irregular structures which were surface rendered in three dimensions and quantified. Operating at a key checkpoint for cellular transport, we suspect this protein may play a larger role in cellular trafficking of many other GPCRs.

#190 - Exploiting the Unique Properties of Carbon Nanohoops for Use in Biological Applications

Otteson C., and Jasti R., University of Oregon

Recently, there has been substantial effort put forth towards utilizing carbon nanomaterials in biological and medicinal applications such as imaging, photo-based therapy methods and drug delivery. This is due in large part to their unique physicochemical properties, including small size and controllable surface functionalization, as well as substantial drug-hosting abilities. However, use of CNMs is often hindered by the heterogeneity inherent to their syntheses, decreasing their efficacy for many applications and leading to research inconsistencies, for example, in toxicity profiles for a molecule like C_{60}. Small molecule

analogues of carbon nanomaterials, [*n*]cycloparaphenylenes ([*n*]CPPs) or carbon nanohoops, are the smallest cross-sections of armchair carbon nanotubes. As small molecules, these nanohoops offer a controlled bottom-up synthetic approach which allows for atomic precision in their fabrication. Nanohoops, therefore, can be tailor-made in ways not yet possible with most carbon nanomaterials. In addition to this, nanohoops have their own set of unique photophysical and supramolecular properties. For example, they can all be excited from a single source due to their common absorbance across sizes, while the fluorescent emission is red shifted as the number of phenylene units decreases. Much like CNMs, carbon nanohoops have a wide variety of applications spanning across different fields. Since their fairly recent isolation, many of these applications are actively being investigated, however those that take place in aqueous media remain relatively underexplored due to challenges with solubility. In 2018 our group published the first example of a biocompatible carbon nanohoop, via sulfonation, for cell imaging.[1] Since then, we have expanded to the synthesis of targeted cell imaging agents based on a meta-CPP scaffold that can be used for one- and two-photon imaging.[2] We have also shown that incorporation of nitrogen into the same meta-CPP scaffold allows for the creation of mechanically interlocked molecules wherein the macrocyclic component is an unusually compact rigid fluorophore. We leveraged this architecture to create a turn-on fluorescent sensor for the hydrosulfide anion (HS^-), a biologically relevant analyte, with great selectivity over other nucleophiles.[3] With this proof of concept, we are working towards rendering these probes biologically compatible as well as expanding their utility to sensing additional biologically relevant species. Finally, we have been exploring modular, scalable routes to water soluble nanohoops to improve upon our initial method, as well as expanding the use of these materials as supramolecular hosts. The incorporation of ester functionality has proven a versatile route to water soluble nanohoops. Further, we have shown that these materials can be used to host unfunctionalized carbon nanohoops (ring-in-ring complexes) as well as larger carbon nanomaterials, such as C_{60}, in water and/or mixed aqueous solutions.

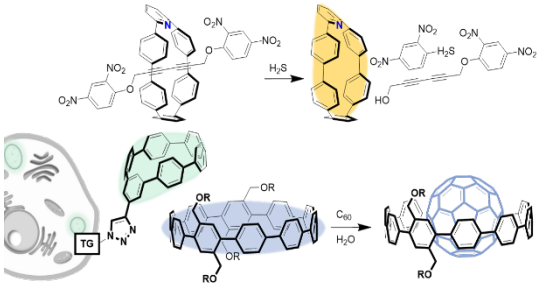

Figure 1. *Nanohoops in water. Left: an intracellularly targeted-nanohoop fluorophore for biological imaging; top: nanohoop-rotaxane hydrosulfide sensor; bottom: a water-soluble [10]CPP capable of hosting C60 in aqueous media.*

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#156 - LCK: AN ENGINEERABLE COMPONENT OF T CELLS

Potter Z. E., Brown J. L., Wei C. T., Fowler D. M., and Maly D. J., University of Washington

Lck's tyrosine kinase activity and scaffolding function are essential for both canonical T cell activation and Chimeric Antigen Receptor (CAR) T cell signaling. Lck uses its kinase domain to phosphorylate the T Cell Receptor (or CAR) immediately following antigen engagement. Then, Lck phosphorylates the kinase ZAP70, and scaffolds a critical interaction between ZAP70 and the Linker for Activation of T cells (LAT), binding each protein using its SH2 and SH3 domains, respectively. Given that other members of the Src Family of Kinases (SFKs)—with identical domain architecture—cannot functionally replace Lck in propagating immunoreceptor signaling, we hypothesized that the intermolecular accessibility of Lck's regulatory SH2 and SH3 domains must be uniquely tuned amongst the SFKs. We used a Deep Mutational Scan (DMS) with ~5,000 single amino acid variants of Lck to gain residue-level information about Lck's SH3/SH2-KD regulatory module. In comparison with a similar DMS dataset collected for Src—another SFK—we find that Lck's SH3/SH2-KD regulatory module plays the prevailing role in regulating Lck's kinase activity even in the absence of inhibitory C-terminal tail phosphorylation. Conversely, Lck's SH4 domain-αF pocket interaction appears to regulate Lck's kinase activity to a lesser extent, compared to Src. This finding is consistent with existing models of Lck's regulation wherein Lck's SH4 domain is bound to co-receptor, and

therefore less available for intramolecular regulation. In addition, given Lck's central role in immune signaling, we reasoned that variants of Lck could be leveraged for clinical utility in next-generation T cell-based therapies. We applied various selective pressures to our DMS assay to identify Lck variants with desired properties that address issues facing current T cell-based therapies including poor solid tumor penetrance, exhaustion, and clinical safety. We are currently validating—and excited to share—the potential of these variants in mammalian cell systems and human T cells, with the vision of using engineered Lck variants in next-generation T cell-based therapies.

#152 - CHEMICAL APPROACHES TO PROBE THE REGULATION OF S-PALMITOYLATION

Qiu T., Kathayat R. S., Cao Y., Azizi S.A., and Dickinson B. C., University of Chicago

S-palmitoylation is an abundant and reversible lipid post-translational modification (PTM) that participates in regulating protein localization, trafficking and therefore has consequences in cellular signaling1,2. We have developed several chemical tools to probe the regulation of S-palmitoylation, including fluorescence-based turn-on depalmitoylation probes (DPPs)3,4,5 and mitochondrial-targeted DPPs (mitoDPPs) to probe "erasers [acyl protein thioesterases (APTs)]" activity in the live cells, fluorescence-based turn-on palmitoylation probes to identify "writers (DHHC)" inhibitor via high-throughput screening, and mitochondrial-targeted APT inhibitor (mitoFP) to study APT function in mitochondria. Using mitoDPPs, we established that there is active S-depalmitoylation in mitochondria, and also showed that mitochondrial antioxidant buffering capacity diminishes on perturbing the APT activity, using either pan-active inhibitors or a new mitochondrial-targeted APT inhibitor6. We demonstrated that ABHD10 is a new member of the S-palmitoylation eraser family. We then identified a key cellular antioxidant protein, peroxiredoxin 5 (PRDX5), as the first target of ABHD10 S-depalmitoylase activity. Furthermore, we showed that S-palmitoylation exclusively occurs at active site cysteine residue in PRDX5, which provides a mechanistic rationale connecting ABHD10-mediated S-depalmitoylation of PRDX5 and its antioxidant capacity. More broadly, expanding the APT family by addition of ABHD10 will help assign more substrates and functions to mitochondrial S-palmitoylation. Additionally, the new mitochondrial-targeted APT inhibitor will help the S-palmitoylation research community to further explore function and regulation of S-depalmitoylation in mitochondria. References. (1) Nat. Rev. Mol. Cell. Biol. 8, 74 (2007). (2) F1000Res. 4, 261 (2015). (3) Nat Chem Biol. 13, 150 (2017). (4) Biochemistry 57, 221 (2018). (5) Nat Commun. 9, 334 (2018). (6) Nat. Chem. Biol. 15, 1232 (2019)

#116 - STRUCTURE-BASED DESIGN OF A POTENT AND SELECTIVE INHIBITOR OF PARP7

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Nucleic acid (NA) sensing—an evolutionarily conserved key component of innate immunity—serves not only as a first line of defense against pathogens, but is also involved in cancer immunosurveillance. Targeting NA sensing in cancer cells is a promising approach for antitumor therapies. PARP7 is a mono—ADP-ribosyl transferase that has emerged as a critical negative regulator of NA sensing in certain cancers. Recent studies show that inhibiting the catalytic activity of PARP7 promotes both intrinsic and immune cell-mediated cancer cell death, and is thus a new clinical target for cancer treatment. Herein, I will describe how we used structure-guided design to generate a potent and selective inhibitor of PARP7 (KMR-206) which exhibits similar biochemical potency against PARP7 as the clinical candidate RBN-2397. We examined NA sensing after treatment of mouse colon carcinoma (CT-26) cells with KMR-206 or RBN-2397 and observed near indistinguishable activation of NA sensing pathway markers. However, we found that KMR-206 and RBN-

2397 inhibition triggered differing levels of PARP7, despite similar biochemical and cellular potencies. Interestingly, the PARP7 protein abundance induced by each inhibitor correlated with differential IFN-β production. These results highlight variable responses to comparable but structurally distinct compounds and are important considerations for current and future therapeutic interventions targeting PARP7.

#144 - HYPERACCURATE RIBOSOMES FOR IMPROVED GENETIC CODE REPROGRAMMING

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The ability to incorporate noncanonical amino acids (ncAAs) has proven to be a very powerful tool in protein labeling/probing and peptide drug discovery. In-vitro translation system like PURE allows intricate manipulation of individual components to tune the translation machinery to push formation of synthetic peptides. When sophisticated ncAAs are in question, it faces multiple challenges put forth by the competing near-cognate readthrough and peptidyl-tRNA dropoff. A very few studies have looked at the effects of competition of near/non- cognate AA-tRNA/EfTu/GTP complexes on speed and accuracy. However, none have been performed in a complex PURE translation environment. Here we use mRNA templates with both N- and C- terminal affinity tags to uncover surprising extent of these competing pathways. We also show that an error restrictive ribosome with mutation in ribosomal protein rpsL, in proximity to the decoding center are more resistant to these competing pathways. Not only that, we also show how this mutant ribosome (mS12) show improvements in yield and accuracy of translation for both the canonical AAs and ncAAs. We explore the use of mS12 in quadruplet suppression experiments where it shows better yield for quadruplet codon readthrough by a qtRNA than the WT ribosomes. However, it does not eliminate triplet codon reading by this tRNA. Along with the versatile PURE translation system, this hyperaccurate ribosome can be a very useful tool to improve the translational fidelity and an attractive tool in the field of synthetic biology.

#172 - ACTIVITY-BASED PROTEIN PROFILING AND TANDEM MASS TAG LABELING ENABLE EFFICIENT, QUANTITATIVE, AND COMPREHENSIVE ORGANOPHOSPHATE TARGET MAPPING ACROSS MAMMALIAN TISSUES

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While organophosphate (OP) poisoning remains a concern in occupational, public, and warfare settings, very few medical countermeasures for treating OP exposure exist. Acute OP toxicity, which can lead to convulsions, seizures, and even death, is a direct result of cholinesterase inhibition. However, multiple lines of evidence point to non-cholinergic mechanisms of action, including inhibition of diverse classes of enzymes such as lipases, proteases, and hydrolases, which may contribute to long-term physiological effects of OP exposure. Considering the broad scope of proteins that may be affected by OPs, efficient tools are needed for a more comprehensive understanding of OP targets and their distribution across tissues. To meet this need, we have combined two powerful chemical proteomics approaches, Activity-Based Protein Profiling (ABPP) and Tandem Mass Tag (TMT) labeling, which allow for efficient sample screening and target quantitation. Using this approach, we have identified a list of both known and novel OP targets in various tissues from mice, rat, rabbit, and guinea pig. In the near future, we will implement our approach in rhesus macaque and human tissues. Such comprehensive receptor mapping, combined with receptor distribution data across different tissues and animals, will help to select the best animal model to guide the development of new therapeutic remedies for OP poisoning.

#106 - GENETICALLY-TARGETED PHOTORELEASE OF ENDOCANNABINOIDS ENABLES OPTICAL CONTROL OF GPR55 IN PANCREATIC BETA-CELLS

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Fatty acid amides (FAAs) are a family of second-messenger lipids that target cannabinoid receptors, and are known mediators of glucose-stimulated insulin

secretion from pancreatic β-cells. Due to the diversity observed in FAA structure and pharmacology, coupled with the expression of at least 3 different cannabinoid G protein-coupled receptors in primary and model β-cells, our understanding of their role is limited by our inability to control their actions in time and space. To investigate the mechanisms by which FAAs regulate β-cell excitability, we developed the Optically-Cleavable Targeted (OCT)-ligand approach, which combines the spatial resolution of self-labeling protein (SNAP-) tags with the temporal control of photocaged ligands. By linking a photocaged FAA to an o-benzylguanine (BG) motif, FAA signaling can be directed towards genetically-defined cellular membranes. We designed a probe to release palmitoylethanolamide (PEA), a GPR55 agonist known to stimulate glucose-stimulated insulin secretion (GSIS). When applied to β-cells, OCT-PEA revealed that plasma membrane GPR55 stimulates β-cell Ca^{2+} activity via phospholipase C. Moving forward, the OCT-ligand approach can be translated to other ligands and receptors, and will open up new experimental possibilities in targeted pharmacology.

#154 - A PROTOCOL FOR PRODUCTION OF ISOTOPICALLY LABELED PROTEINS WITH SITE-SPECIFIC PHOSPHOSERINE INCORPORATION

Vesely C. H., Reardon P. N., Yu Z., Barbar E., Mehl R. A., and Cooley R. B., Oregon State University

More than two-thirds of proteins in the human proteome undergo reversible phosphorylation and of these, nearly 80% were identified at serine residues. This post-translational modification allows proteins to adopt new conformations to inhibit protein function, activate catalytic activity, form new protein-protein interactions, or dissociate protein complexes. Most of these phosphoserine (pSer) sites are located with disordered loops or regions of proteins, and so the ability to produce proteins with site-specific pSer isotopically labeled with ^{15}N (and ^{13}C) for Nuclear Magnetic Resonance (NMR) studies would prove very powerful in our efforts to uncover molecular mechanisms of protein regulation by phosphorylation. In 2015, a high-efficiency genetic code expansion (GCE) system was developed to translationally install pSer. Yet, no one has successfully adapted this GCE system to produce isotopically labeled, intrinsically disordered proteins (IDPs) with site-specific pSer incorporation because the *E. coli* pSer protein expression host cannot grow in minimal media. Here, we overcome this problem and develop and optimize an expression methodology to produce isotopically labeled proteins with homogenous, site-specific phosphorylation. With these methods we achieve ≥10 mg per liter of culture of homogenously labeled, phosphorylated super folder green fluorescent protein (sfGFP), and demonstrate its quality and stability with successful NMR experiments. As a biologically relevant example, we also produce the intrinsically disordered region (IDR) of the SARS-COV-2 Nucleocapsid protein isotopically labeled with pSer at a key site that is critical for viral infection activities but for which the molecular mechanism of functional change remains unknown. Thus, this efficient and low-cost methodology opens the door to easy access of isotopically labeled pSer containing proteins for 2D and 3D NMR analysis.

#102 - CHEMICAL PHOTOSWITCHES TO REVERSIBLY MANIPULATE CANNABINOID RECEPTOR ACTIVITY

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Frank J., Oregon Health & Science University

The cannabinoid receptors CB1 and CB2 are inhibitory class A GPCRs and classic receptors of the endocannabinoid system. CB1 is most abundantly expressed in the central nervous system (CNS), while CB2 is expressed predominately in the periphery by immune cells. Both receptors have disease relevance and serve as promising therapeutic targets. For example, CB2 expression is upregulated in the CNS under conditions of injury, inflammation, and neurodegenerative diseases. Exogenous

cannabinoid ligands such as tetrahydrocannabinol (THC) activate both CB1 and CB2, while synthetic cannabinoid ligands like HU308 selectively activate CB2. The hydrophobicity of cannabinoid ligands poses diffusion limitations which challenge our ability to modulate cellular excitability. To this end, we developed photoswitchable cannabinoids whose signaling activities can be tuned in a light-dependent manner. Photoswitchable analogs of THC and HU308 were synthesized to place both CB1 and CB2 signaling under optical control. The probes were evaluated in cultured -cells and neurons, which shed light on novel effector pathways by which these GPCRs can affect excitability. Future applications of these probes in primary cells or in vivo will be useful to interrogate the role of CB1 and CB2 in cellular signaling and disease.

#136 - Proteomic Characterization of Phagocytic Primary Human Monocyte-Derived Macrophages

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Macrophages play a vital role in the innate immune system, identifying and destroying unwanted cells. However, a comprehensive understanding of human monocyte-derived macrophage protein abundance has been difficult to attain due to technological limitations. In this study we developed methods to derive human macrophages and prepare them for mass spectrometry analysis in order to more-deeply understand the proteomic consequences of macrophage stimulation. Interferon gamma (IF-g), an immune stimulating cytokine, was used to induce macrophage activation, increasing phagocytosis of cancer cells by 2-fold. These conditions were used to perform comparative shotgun proteomics between the macrophage resting state and stimulation resulting in increased phagocytic ability. In addition to whole protein changes, we evaluated active protein synthesis by treating cells with the methionine surrogate probe homopropargylglycine (HPG). Our analysis revealed that macrophages bias their protein production toward biological processes associated with phagocytosis and antigen processing in response to IF-g treatment. We confirmed our findings by antibody-based Western blotting experiments, validating both previously reported and novel proteins of interest. In addition, we saw increased rates of HPG incorporation during IF-g treatment, suggesting protein synthesis rates are altered during stimulation events. Together our findings provide the most comprehensive proteomic insight to date into primary human macrophages. We anticipate that this data can be used as launchpoint to generate new hypotheses about innate immune function.

#198 - Measuring RNA Tertiary Contacts in SARS-CoV-2

Willis D., Kimmett E., and DeRose V. J., 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.

#204 - Inhibition of RNA Pol I activity by nucleolar stress-inducing Pt(II) compounds.

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Platinum(II)-based chemotherapeutics are an important class of compounds with broad use in anticancer regimens. Canonically, these compounds are thought to interfere with DNA replication by forming cross-links with genomic DNA. In rapidly dividing cancer cells, which often lack fully functional DNA damage repair mechanisms, these crosslinks induce a DNA Damage Response (DDR) ultimately leading to apoptosis. More recently our lab and others have found that a subset of Pt(II)-based compounds, including the FDA-approved chemotherapeutic oxaliplatin, induces cell death primarily by disruption of ribosome biogenesis. In recent work, we have shown that oxaliplatin disrupts ribosome biogenesis though inhibiting rRNA transcription, and that

this correlates with morphological changes in nucleolar structure. The specific targets or mechanism for inhibition of RNA Pol I activity are currently under investigation. To gain further insight into the inhibition of rRNA transcription by oxaliplatin, we are performing ChIP-based assays to measure occupancy of RNA Pol I across different regions of the rDNA sequence. Results from these studies will confirm the influence of oxaliplatin on RNA Pol I, and determine whether Pol I initiation or elongation are affected by this platinum compound. Addition of platinum compounds to the currently limited number of specific RNA Pol I inhibitors is important as nucleolar stress induction is an emerging area for anticancer therapeutics.

#158 - GENETIC ENCODING OF PROTEIN TYROSINE NITRATION REVEALS THE IMPACT OF 14-3-3 OXIDATIVE DAMAGE ON CLIENT BINDING

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Oxidative protein tyrosine nitration has been established as a physiologically relevant post-translational modification found throughout mammalian proteomes, particularly when under oxidative stress conditions. Although tyrosine nitration is such a pervasive post-translational modification, very little is known about how it serves to modify protein function and how these modifications correlate with disease. The 14-3-3 family of proteins is critical to maintaining functional cellular phosphorylation signaling systems; and they are found to be nitrated at critical tyrosine residues under physiological and immune-stimulatory conditions. To better understand how tyrosine nitration affects 14-3-3 structure and function, we genetically encoded 3-nitrotyrosine site-specifically into these critical tyrosine residues found to be nitrated, and encoded phosphoserine into 14-3-3 binding partners. Using X-ray crystallography and other biochemical techniques we elucidate the structural and functional consequences of tyrosine nitration in 14-3-3 proteins. Our data suggests that 14-3-3 nitration will impact phosphorylated client regulation inside cells and provides a link between 14-3-3 oxidative damage and phosphorylation signaling dysregulation.

#151 - BIOSENSOR OF ENDOLYSOSOMAL FUNCTION BY LIGAND DIRECTED LABELING OF THE DELTA OPIOID RECEPTOR WITH OREGON GREEN 488

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Membrane proteins traffic through the cell in highly controlled manners with important downstream consequences. For example, activation of one GPCR, Delta Opioid Receptor (DOR), leads to trafficking from the plasma membrane to the lysosome for degradation, while the related Mu Opioid Receptor (MOR) is recycled back to the plasma membrane. To adapt ligand directed labeling of opioid receptors to become a biosensor for endolysosomal maturation and health, two approaches could be taken, a pH sensitive approach or a proteolysis sensitive approach. The pH approach leverages the pH gradient through the endolysosome and a pH sensitive fluorophore to gain or lose signal as pH decreases, while the proteolysis approach uses a hydrophobic fluorophores which can pass out of the membrane after cleavage from the protein. We began by conjugating Naltexamine-Acyl Imidazole to Oregon Green 488, a pH sensitive slightly hydrophobic fluorophore, to create NAI-OG488. In applying the technology to HEK293 cells stably expressing DOR, we found that over long time courses, more fluorescence was lost than could be explained with the published OG488 pKa or the accepted pH range of the lysosome. Additionally, when using NAI-AF488 to compare to a pH insensitive, hydrophilic dye, no fluorescence change was noticed over the same time course. We hypothesized three possible causes of the OG488 fluorescence decrease – the first that acidification of the lysosome caused the decreased fluorescence. Alternatively, OG488 could be cleaved off of the protein and either sequestered in the membrane, self-quenching, or able to pass through the membranes, leaving the cell. To test lysosomal the acidification hypothesis, we deacidified lysosomes before agonist stimulation with BafA1 or after agonist stimulation with Chloroquine. To test for membrane sequestration and quenching, cells were lysed with TritonX to free any trapped dye. None of the above approaches rescued fluorescence, thus the remaining scenario of OG488 being cleaved from the receptor and escaping the cell is the most likely.

#127 - DEVELOPMENT OF AN ACRYLAMIDE-BASED DHHC INHIBITOR TO REGULATE ERK ACYLATION AND ACTIVITY

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Protein *S*-acylation, an enzymatically modulated lipid post-translational modification (PTM), is frequent and consequential; at the cellular level, it regulates protein subcellular trafficking and signaling activity, and at the organismal level, has been implicated in neoplastic, neurologic, and autoimmune disease. However, the impact of dynamic *S*-acylation for many proteins remains unknown, in part due to lack of inhibitors for the enzymes that catalyze lipid addition, the DHHC family proteins. Not only is the state-of-the-art pan-DHHC inhibitor, 2-bromopalmitate (2BP), toxic and poorly potent, it also inhibits the *S*-acylation eraser proteins, the acyl protein thioesterases (APTs). Here, we report the synthesis and characterization of **CMA**, a DHHC inhibitor with improved potency and selectivity. We then use it to identify and characterize dynamic *S*-acylation as a novel PTM for the extracellular signal-regulated kinase (ERK1/2), a substrate whose *S*-acylation is resistant to 2BP.

As an α-halogenated fatty acid, 2BP reacts with a range of nucleophilic amino acids and forms reactive acyl CoA intermediates *in cellulo*. We therefore proposed to exchange the α-halogen with an acrylamide, a warhead that reacts with cysteine over serine residues and would not undergo metabolic conversion. Synthesis and screening of a panel of acrylamide-containing lipids revealed **CMA** as a potent DHHC20 inhibitor *in vitro*, with the ability to significantly decease both substrate and global protein *S*-acylation *in cellulo*. Competitive activity-based protein profiling (ABPP) demonstrated that **CMA** engages directly with a panel of DHHCs in cells.

Moreover, **CMA** is less toxic than 2BP, does not inhibit eraser APT activity, and is able to effect cellular responses previously reported to result from loss of *S*-acylation. These results position the acrylamide as an inhibitory scaffold for DHHC family proteins.

We next used CMA to probe the effects of perturbing palmitoylation on epidermal growth factor (EGF)-triggered signal transduction. Characterization of disruptions along this signaling cascade enabled the identification of a previously undescribed acylated protein, the extracellular signal-regulated kinase (ERK1/2), whose S-acylation is resistant to 2BP but responsive to CMA. We then use a multi-pronged approach to describe the molecular determinants of ERK1/2 acylation and discover crosstalk between ERK1/2 phosphorylation and palmitoylation, as well as lipidation-dependent changes in its transcriptional program. Together, these results suggest the criticality of S-acylation in regulating the activation and downstream behavior of this key effector protein. Finally, we also examine ERK1/2 S-acylation in a mouse model of metabolic syndrome, correlating changes in its lipidation levels with alterations in writer/eraser expression and solidifying the link between ERK1/2 lipidation and organismal health.

In sum, this work reveals the potential of acrylamide-based DHHC family inhibitors and emphasizes that much remains to be discovered regarding the significance of dynamic S-acylation in regulating protein activity and cellular events.

#185 - Dual Encoding and Labeling of Proteins in Living Cells

Bednar R. M., Jana S., Kuppa S.. Franklin R. Beckman J., Antony E., Cooley R. B., and Mehl R. A., Oregon State University

A general, two-step approach to achieving site-specific dual labeling of proteins in their native environment that relies on dual encoding of two distinct noncanonical amino acids and their subsequent mutually bioorthogonal labeling is presented herein. We demonstrate for the first time that tetrazine- and azide-bearing noncanonical amino acids can be simultaneously encoded into a single protein and that subsequent labeling with a strained alkene and dibenzoannulated cyclooctyne, respectively, can be performed orthogonally in living Escherichia coli cells. Using this Dual Encoding and Labeling (DEAL) approach, we further demonstrate the feasibility of several abilities, including: site-specific dual labeling of several proteins for FRET, topologically-defined protein-protein cross-linking, and intramolecular protein stapling.

#125 - Intracellular Targeting of RNA and RNA-Protein Interactions via Biomimetic Triplex Hybridization with bPNAs

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Bifacial Peptide Nucleic Acids (bPNAs) utilize synthetic triazine bases to form triplex hybrid stems with oligo T/U domains that are biomimetic of native triple stranded structures formed from UAU base-triples. Indeed, bPNAs can displace oligo-A strands from U-rich internal loops (URILs) to form a synthetic triplex hybrid. We report herein low molecular weight (<1 kD) bPNAs capable of selective intracellular labeling of RNAs containing U-rich internal loops (URILs). Pre-structured URILs, which are relatively rare, were found to be particularly selective for bPNA binding even among other native U-rich transcripts. This enables selective targeting, pulldown and triggered degradation of native URILs, as found in lncRNAs NEAT1 and MALAT1 (NEAT2). Furthermore, engineered URILs enable fluorogenic bPNA labeling of RNAs of interest and associated RNA-protein complexes. We anticipate that this and related bPNA targeting strategies will be useful for interrogation of intracellular RNA interactions.

#211 - A high-throughput fluorescent turn-on assay for inhibitors of DHHC family proteins

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As the “writer” enzymes of protein S-acylation, a dynamic and functionally significant posttranslational modification (PTM), DHHC family proteins have emerged in the last decade as both key modulators of cellular homeostasis and as drivers of neoplastic, autoimmune, metabolic, and neurological

pathologies. Currently, biological and clinical discovery is hampered by the limitations of existing DHHC family inhibitors, which possess poor physicochemical properties and off-target profiles. However, progress in identifying new inhibitory scaffolds has been meager, in part due to a lack of robust in vitro assays suitable for high-throughput screening (HTS). Here, we report the development of palmitoyl transferase probes (PTPs), a novel family of turn-on profluorescent molecules that mimic the palmitoyl-CoA substrate of DHHC proteins. We use the PTPs to develop and validate an assay with an excellent Z'-factor for HTS. We then perform a pilot screen of 1,687 acrylamide-based molecules against zDHHC20, establishing the PTP-based HTS assay as a platform for the discovery of improved DHHC family inhibitors.

#115 - RNA-BASED TRANSLATIONAL ACTIVATORS FOR TARGETED ENDOGENOUS GENE UPREGULATION

Cao, Y., Liu H., Lu S. S., Govind A. P., Dickinson B. C., The University of Chicago

Technologies capable of activating the expression of genes in a programmable way offer a strategy to develop therapeutics for diseases caused by gene deficiency. Here, I will present "translation-activating RNAs" (taRNAs), a bifunctional RNA-based molecular platform that binds to a target mRNA of interest and directly upregulates protein production from it. We engineer taRNAs from a variety of natural RNA IRES elements and demonstrate gene activation from a suite of target mRNAs. We develop a minimized 125-nt taRNA, identify two different cellular components responsible for taRNA activity, and validate the technology by activating haploinsufficiency disease-related targets, SYNGAP1 and PMP22. Finally, we show that taRNAs can be delivered as RNA molecules by lipid nanoparticles (LNPs) in cell lines, primary neurons, and to mouse liver in vivo. taRNAs provide a versatile nucleic acid-based platform to programmably upregulate protein production from endogenous mRNAs, opening up new possibilities for therapeutic RNA design.

#145 - PERMAPHOS: AUTONOMOUS SYNTHESIS OF FUNCTIONAL, PERMANENTLY PHOSPHORYLATED PROTEINS

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Cooley R. B., Oregon State University

Installing stable, functional mimics of phosphorylated amino acids into proteins offers a powerful strategy to study mechanisms of protein regulation. Previously, a genetic code expansion (GCE) system was developed to translationally install non-hydrolyzable phosphoserine (nhpSer), with the γ-oxygen replaced with a CH_2 group, site-specifically into proteins but it has seen limited usage. Here, we achieve a 40-fold improvement in this system by engineering into *Escherichia coli* a biosynthetic pathway that produces nhpSer from the central metabolite phosphoenolpyruvate. Using this "PermaPhos" system – an autonomous *E. coli* expression system for incorporating nhpSer into target proteins – we show that nhpSer faithfully mimics the effects of phosphoserine in three stringent test cases: promoting 14-3-3/client complexation, disrupting 14-3-3 dimers, and activating GSK3β phosphorylation of the SARS-CoV-2 nucleocapsid protein. This facile access to milligram quantities of nhpSer-containing proteins from *E. coli* overcomes hydrolysis issues associated with recombinant production of pSer proteins. It also opens to the door to studying phospho-proteins in systems where exposure to phosphatases is expected, such as incubation in cell lysate for pulldown interaction studies, transfection into eukaryotic cells, for studying phosphatase regulation by phosphorylation, and antibody development. PermaPhos should allow nhpSer to replace Asp and Glu as the go-to stable pSer phosphomimetic for proteins produced in *E. coli.*

#163 - SYNTHESIS AND ANTI-CANCER EVALUATION OF ACYLATED MONOAMINOPYRROLOQUINAZOLINES

Dhoro F., Dannar A., Chao B., Li B., and Xiao X., Oregon Health and Science University

The privileged pyrroloquinazoline scaffold, 7*H*-pyrrolo[3,2-*f*]quinazoline-1,3-diamine, was originally synthesized as an antifolate. Its derivatives have been shown to possess a variety of biological activities that include antibacterial, anticancer and antiparasitic effects. We have previously developed methods to regioselectively mono-N-acylate the three nucleophilic nitrogens of 7*H*-pyrrolo[3,2-*f*]quinazoline-1,3-diamine. From the library of mono-*N*-acylated 1,3-diaminopyrroloquinazolines synthesized, compounds possessing an acyl group at *N*-3 were found to possess potent antiproliferative activities in breast cancer cells. Furthermore, we identified the first small molecule called **LBL1** to bind nuclear lamins from this focused library of compounds. Recently, we found that deletion of *N*-1 amino group in LBL1 led to significant enhancement of its anti-cancer activity. These results suggested that removing the N-1 primary amino group in 1,3-diaminopyrroloquinazolines could lead to chemical entities with improved anticancer activity. In this presentation, a series of mono-acylaminopyrroloquinazolines was synthesized and their potential anticancer activities were evaluated. Indeed, we identified improved compounds with single digit to double digit nanomolar (6-10 nM) potency in breast cancer cells. We conclude that these mono-acylaminopyrroloquinazolines represent novel anti-cancer agents.

#175 - THE HOLY GRAIL OF BIOCONJUGATION IS WITHIN REACH AS WE MARRY QUANTITATIVE LABELING WITH FAST REACTION RATES

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Jana S., Oregon State University

Mbengi L., Oregon Health & Sciences University

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Mehl R. A.,, Oregon State University

Advancements in protein bioconjugations labeling rates and quantitative labeling provided exciting new options for biomaterial production and the study of biomolecule function and localization. Quantitative Protein labeling through genetic code expansion is limited by (1) encoding fidelity, (2) slow reaction speed, (3) reactant stability (4) product stability. Many reactions are too slow or unstable to provide quantitative ligations on an experimentally relevant time scale. Previously, we site-specifically encoded highly stable 1,2,4,5-tetrazine noncanonical amino acids and displayed rapid, high-yielding reactions which opened access to low reactant concentrations. This Tet-labeling technology has enabled substoichiometric labeling of proteins on a rapid time scale but can experience redox dependent reactivity. Unfortunately, a small percentage of unreactive protein is observed regardless of reaction time or partner reactant concentration. Using reactivity-dependent mobility shift assays we evaluated our Tet-v2.0 encoding technology and identified that the reactivity of our system is not hindered by decomposition or redox state, rather, by genetic misincorporation of natural amino acids due to near-cognate suppression. To overcome this limitation and achieve quantitative reactivity we evolved a more catalytically efficient 2nd generation Tet-v2.0 aaRS that utilizes a new Tet-v2.0 variant and then optimized levels of genetic code expansion machinery to enhance TAG site suppression. Our approach exemplifies how quantitative labeling with high reaction rates is possible if low concentration byproducts are characterized and systematically removed.

#167 - DEVELOPMENT OF CARDIO- AND IMMUNE-SAFE TYROSINE KINASE INHIBITORS FOR BCR-ABL

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Eide C., Oregon Health & Science University

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Dheeraj A., Oregon Health & Science University

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Development of tyrosine kinase inhibitors (TKIs) that target the BCR-ABL oncogene has been successful in the treatment of chronic myeloid leukemia (CML) and/or acute lymphoblastic leukemia (ALL). However, clinically-utilized inhibitors are limited by drug resistance and toxicity. Mutations within the kinase domain of BCR-ABL constitute the most frequent mechanism of drug resistance. Particularly, the T315I "gatekeeper" mutation that occurs in over 20% of CML patients is resistant to all the approved BCR-ABL inhibitors, except ponatinib. Ponatinib, a third-generation inhibitor, has demonstrated excellent efficacy against both wild type and mutant BCR-ABL kinase, including the T315I "gatekeeper" mutation. However, ponatinib is one of the most cardiotoxic FDA-approved TKIs. Therefore, there is a need to discover safer BCR-ABL inhibitors that work against both wild type and the T315I mutation. Recently, using a structure guided approach, our lab has discovered a novel series of potent BCR-ABL inhibitors. To identify cardiac-safe analogues, our drug design paradigm was coupled to iPSC-cardiomyocyte (iPSC-CM) models. Systematic SAR studies discovered two inhibitors 1 and 2, which significantly inhibit the kinase activity of both native BCR-ABL and the T315I mutant. Our inhibitors are the most cardiac-safe TKIs reported to date, and may effectively treat CML patients with the T315I mutation. In addition to cardiotoxicity, TKIs are known to impact immune function, including through disruption of T-cell receptor (TCR) signaling. The present study focused on T-cell proliferation and cytokine responses in the presence of clinical TKIs and our new inhibitors. Ponatinib treatment results in defective expansion and response of T cells to stimulation, which would stunt their capacity to address both infection and cancer-related threats. This effect also prevents combination treatment synergy between TKIs and checkpoint inhibitors. Our inhibitors show reduced toxicity to hematopoetic stem cells as well as activated T-cells. In comparison to clinically-utilized TKIs, our inhibitors allow T-cells to proliferate and release normal cytokines in response to TCR stimulation.

#155 - STRUCTURAL INSIGHTS INTO THE IMPROVED EFFICIENCY OF AN EVOLVED METHANOMETHYLOPHILUS ALVUS AMINO-ACYL TRNA SYNTHETASE FOR A FLUORESCENT NONCANONICAL AMINO ACID

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Karplus P. A., Oregon State University

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Genetic code expansion (GCE) is a technology that allows site-specific incorporation of noncanonical amino acids (ncAAs) into recombinant proteins at amber stop codons using engineered translational machinery. The most utilized systems make use of reengineered pyrrolysine amino-acyl tRNA synthetase (aaRS)/tRNA pairs from the archaeal species *Methanosarcina barkeri (Mb)* and *Methanosarcina mazei (Mm*) because they are natural amber stop codon suppressors and are orthogonal to prokaryotic and eukaryotic translational systems. Unfortunately, the efficacy of these aaRSs for *in vivo* protein expression and *in vitro* characterization is limited by the presence of a nuclear localization sequence, low solubility, and a tendency to be proteolyzed. A recently developed pyrrolysine aaRS/tRNA pair from *Methanomethylophilus alvus (Ma)* overcomes these issues as the aaRS is highly soluble, stable and is localized primarily in the cytoplasm. To adopt the *Ma* system for broad use, understanding how best to evolve its specificity for ncAAs is needed. Typically, an orthogonal aaRS/tRNA pair is engineered to be selective for an ncAA by directed evolution. However, the tertiary structure of the *Mm/Mb* catalytic domain and the *Ma* aaRS are highly similar so it has been proposed that active sites evolved in *Mm/Mb* for ncAAs can be easily transplanted into *Ma* to confer specificity to the ncAA, thereby hastening the engineering process by avoiding further evolution. While this approach has proven successful for some ncAAs, such as lysine derivatives, work in our lab has shown that active site transplants (AST) for aromatic ncAAs produce functional, but very inefficient *Ma* aaRSs. Instead, we find that evolution of *Ma* aaRSs produce much more efficient aaRS/tRNA pairs than ASTs. This was

particularly apparent for *Ma* aaRSs able to incorporate the amino acid acridone, a fluorescent ncAA, with the evolved aaRS being about 8-fold better than the AST. Here, we present crystal structures of the *Ma* acridone AST and evolved aaRS with acridone and ATP bound to 1.5 Å resolution. These structures clarify the cause for improved performance of the evolved *Ma* aaRS compared to the AST. This constitutes the first structures of the *Ma* aaRS with ligands and will be used to guide future engineering of the aaRS for diverse ncAAs including acridone derivatives with desirable fluorescent properties.

#143 - THE ADVANTAGES OF FULLY MODIFIED tRNAs FOR IN VITRO TRANSLATION WITH NON-CANONICAL AMINO ACIDS.

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In vitro Genetic code reprogramming for the introduction of non-canonical amino acids (ncAAs) enables the creation of diverse, drug-like macrocyclic peptide libraries. However, many ncAAs are much poorer substrates for the translation apparatus than their canonical counterparts. In addition, most in vitro translation experiments with ncAAs utilize in vitro transcribed tRNAs which lack the posttranscriptional tRNA modifications that are present in vivo. Many of these modifications are known to improve translation efficiency and fidelity. We therefore surmised that fully modified tRNAs may prove beneficial for in vitro translation with ncAAs. To this end, we developed a new, simple, and scalable method for isolation of homogeneous tRNA isoacceptors from E. coli total tRNA. Using the five leucine isoacceptors as a case study, we compared the efficiency of translation and the codon reading preferences of fully modified vs. in vitro transcribed tRNAs. In every case, fully modified tRNAs were better substrates for the translation apparatus. This higher efficiency proved beneficial for breaking the degeneracy of the leucine codon box with various ncAAs. Based on these results, we expect fully modified tRNAs to be an enabling technology for the maximal breaking of codon degeneracy with ncAAs, permitting the creation of short, yet diverse macrocyclic peptide libraries via mRNA display.

#197 - A NOVEL METHOD FOR INVESTIGATING THE MECHANISM OF ANTIBODY INTERNALIZATION IN PARANEOPLASTIC SYNDROMES

Hecht R., Shi H., Duvoisin R., and Morgans C., Oregon Health & Science University

Melanoma-associated retinopathy (MAR) is a paraneoplastic syndrome that causes visual impairment in some cutaneous malignant melanoma (CMM) patients. The visual symptoms of MAR, which include poor night vision, diminished contrast sensitivity, and shimmer vision (photopsia), are the result of an off-target immune response against specific neurons in the retina called ON-bipolar cells (ON-BCs). During the anti-cancer immune response to mitigate melanoma tumor progression, B cells produce “autoantibodies” against melanocyte antigens. Since ON-BCs and melanocytes both express the channel protein Transient Receptor Potential Cation Channel Subfamily M Member 1 (TRPM1), anti-TRPM1 autoantibodies produced against the tumor can cross-react with TRPM1 in ON-BCs, compromising their ability to depolarize in response to light stimuli which results in the visual symptoms of MAR. While it is well-established that these autoantibodies bind an epitope on the intracellular domain of TRPM1, the mechanism by which they penetrate ON-BCs to access this intracellular site remains unknown. Previously, our lab demonstrated that cultured ON-BCs will internalize antibodies in their media. Here we present a novel method for observing antibody uptake in live retinal slices that provides a straightforward approach for determining the cellular mechanism governing antibody internalization in ON-BCs. Improving on cell culture models, our live, agar-embedded sections of mouse retina provide a physiologically relevant environment that preserves endogenous tissue structure and cellular connectivity. Under the proper conditions, these slices can be maintained for several hours post-dissection and are well-suited to drug treatment, allowing for direct manipulation of cellular functions for elucidating uptake mechanisms. Numerous slices can be prepared from each mouse eye, offering simultaneous probing of multiple conditions using a single animal. Furthermore, this strategy can be easily adapted to other tissues, such as the brain, for study of autoantibody internalization in other

paraneoplastic syndromes with intracellular target antigens. A mechanistic understanding of antibody internalization in MAR may yield broader insight into similar paraneoplastic syndromes and present an opportunity for therapeutic intervention across a range of burdensome human diseases.

#113 - UNTANGLING THE ROLE OF ZINC IN THE MAMMALIAN CELL CYCLE AND DNA DAMAGE RESPONSE

Holtzen S., Steward L., Kainov J., Navid E., and Palmer A., University of Colorado Boulder

Zinc is an essential micronutrient that is required for all forms of life. Approximately one in ten human proteins possesses a zinc binding site, and as such, it plays diverse roles as a structural and catalytic cofactor. Zinc also plays a role in informing the proliferation-quiescence decision in mammalian cells. Several studies from the early 2000s have shown that moderate zinc deficiency affects proliferation and activates the DNA damage response, but only recently has this phenomenon been characterized at a single-cell level. Using a CDK2 activity sensor, our lab found that zinc deficiency causes asynchronously cycling mammary epithelial cells to bifurcate in cell fate after division. In the first population, cells enter a resting quiescent state. In the second, cells commit to another cell cycle, only to stall in S-phase. After 24 hours, zinc deficient cells show increased DNA damage and impaired DNA synthesis relative to zinc replete cells. Since this observation was made after 24h of zinc deficiency, it is crucial that we investigate the temporal dynamics of DNA damage relative to zinc withdrawal and as a function of the cell cycle. We used a combination of live- and fixed-cell imaging to identify the series of events that lead to this bifurcation. Preliminary evidence suggests that DNA damage appears as soon as four hours after removal of zinc, implying that zinc deficiency-induced DNA damage occurs prior to the decision to route to quiescence or enter S-phase. To further investigate this phenotype, we are tracking DNA damage in live cells using a sensor that forms puncta upon induction of double-stranded breaks. Our goal is to define the appearance, disappearance, residence time, and puncta number as a function of time relative to other cellular signals. These investigations into zinc's role in genomic stability will address a decades-long knowledge gap in the field of zinc and cell cycle biology.

#193 - 4D IMAGING OF CELL DIVISIONS IN BARNACLES TO UNCOVER THE ANCESTRAL MODE OF CRUSTACEAN EMBRYOGENESIS

Jezuit E., and van Dassow G., University of Oregon

Crustaceans form one of the largest, most diverse animal groups, yet there is no clear model for the ancestral mode of crustacean development. Classically, crustaceans were grouped with annelids, which are spiralians. All classical crustacean embryology therefore assumed that cell cleavage, lineage, and fate specification were somehow derived versions of the spiralian stereotype. Molecular phylogenetics overturned this view by placing crustaceans within Ecdysozoa, no closer to spiralians than other animals (late 1990s).

Furthermore, molecular phylogenetics now places insects, which includes one of the most important model organisms for developmental biology, *Drosophila melanogaster*, amongst crustaceans. Any outline of the evolution of development requires stepping stones along the Tree of Life to connect insects to the rest of the animal kingdom.

If crustacean embryos aren't developing according to a variant of the spiralian plan, what are they doing? We need an experimental model that is a good proxy for "primitive" crustacean development.

Barnacles retain developmental traits thought to be primitive to crustaceans. Fertilized barnacle eggs undergo overt polarization followed by stereotyped oriented asymmetric cell divisions, whereafter, upon reaching a relatively small cell number, they proceed through gastrulation and then commence organogenesis and appendage formation.

While rejecting the spiralian affinity, we seek experimental evidence to support the hypothesis that the barnacle embryo exhibits a consistent linkage between cell lineage and fate specification, mediated by precocious cell polarization and oriented division.

Here I present my work towards determining if the barnacle embryonic cleavage pattern has a consistent relation with cell fate. To accomplish this goal I am both developing and adapting modern/genetic techniques to answer

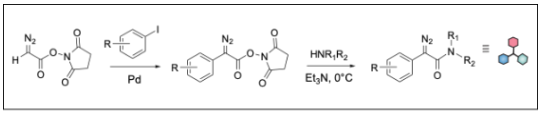
focused questions about the nature of barnacle embryogenesis. The research strategies I use include labeling and tracking of individual blastomeres from cleavage through gastrulation, imaging of both fixed and live specimens using RNA and protein probes for microtubules as well as actin and cell membranes, and perturbations of developmental processes (mechanical, chemical, and in the future, genetic).

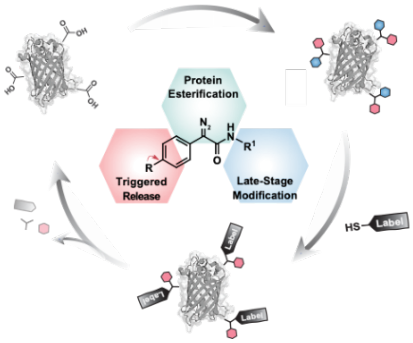
#133 - A Chemoselective Late-Stage Protein Modification for Traceless Cytosolic Delivery

Jun J. V., Petri Y., Erickson L., and Ronald T. Raines R. T., Massachusetts Institute of Technology

While the biological roles of proteins are being discovered at a remarkable pace, the number of FDA-approved biological drugs is significantly lower than that of small molecules. The power and impact of protein therapeutics are substantially undermined because of the fundamental limitation: proteins cannot spontaneously cross the membrane. Conventional delivery techniques fail to address this fundamental problem in that protein cargo is predominantly delivered into cells via endocytosis, leading to degradation pathways. Thus, the ability to modulate protein surface to interrogate factors important for cell permeability is highly desired in both biological research and protein therapeutics. Addressing the fundamental limitation, we developed a bioreversible esterification strategy to endow proteins with the ability to directly enter the cytosol of human cells. Specifically, we show that the library of α-aryl-α-diazoacetamides can esterify carboxyl groups [1] in proteins, enabling their delivery across cellular membranes. The ensuing esters are substrates for intracellular esterases.

To further expand the utility of α-aryl-α-diazoacetamides in protein delivery applications, we developed a more general and modular probe for reversible protein modification [2]. Our probe consists of a diazo moiety for protein conjugation, a thiol-reactive group for late-stage functional diversification, and a self-immolative carbonate group to promote traceless release from the protein. We showed that our probe can generate a diverse set of protein conjugates modified with cell-penetrating peptides, targeting ligands, or PEG under mild conditions. Our strategy represents a significant advance over previous reversible strategies because protein mutagenesis is not required, modifications are done under mild conditions, the probe is synthetically accessible, and most importantly, our strategy is potentially compatible with virtually any protein of interest. Therefore, this strategy implements a traceless means to deliver native proteins into the cytosol of live cells for applications in the laboratory and clinic.





[1] **Jun, J. V.;** Raines, R. T., Two-Step Synthesis of α-Aryl-α-diazoamides as Modular Bioreversible Labels. *Organic Letters* **2021**, 23, 3110-3114.
[2] **Jun, J. V.;** # Petri, Y.; # Erickson, L. W.; Raines, R. T., A Chemoselective Late-Stage Modification for Traceless Protein Delivery *(Manuscript in Prep)* #*equal contributors*

#149 - Monitoring Glycolytic Dynamics in Single Cells Using a Fluorescent Biosensor for Fructose 1,6-Bisphosphate

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Fructose 1,6-bisphopshate (FBP) is a glycolytic intermediate that exhibits large changes in concentration that track with changes in flux through the pathway. The dependance of FBP concentration on glycolytic flux makes this metabolite an intriguing target for live-cell measurements. The B. subtilis transcriptional regulator CggR, is known to bind FBP but has not yet been used to construct a biosensor with rapid kinetics. Using a high-throughput functional assay we screened libraries of candidate biosensors consisting of a circularly permuted fluorescent protein inserted into CggR with variable linkers connecting the two domains. By efficiently characterizing the brightness and dynamic range for many sequence variants in parallel, we were able to discover multiple high-dynamic range FBP biosensors with diverse properties. The top variant, named HYlight, was evaluated in pancreatic beta cells and found capable of uncovering aspects of glycolytic regulation, dynamics and heterogeneity. Given the conservation and importance of glycolysis, we anticipate this new FBP biosensor will be broadly useful for studying metabolism in a variety of tissues and organisms.

#171 - Development of an activity-based probe for streptococcal virulence factor C5a peptidase

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A highly conserved surface bound enzyme, C5a peptidase, has been identified as an important virulence factor in Group A, B, C and G streptococcus. C5a peptidase acts as an adhesion factor during the invasion of the host by binding integrin, fibronectin, and epithelial cells. Additionally, it plays a key role in human immune system evasion by specifically cleaving and thus inactivating the human complement-derived chemotaxin, C5a, which is a 74 amino acid containing peptide. The presentation will include development of a selective activity-based probe (ABP) based on C5a peptidase's specific C5a cleaving trait and a simple gel-based assay to characterize C5a peptidase activity in whole cells and subsequent inhibition of activity by the probe. Furthermore, selectivity of the probe for C5a peptidase is shown using C5a peptidase expressing pathogenic Streptococci, tunable C5a peptidase over expressing E. coli model and mixed cultures of pathogenic Streptococci in the presence of a non-pathogenic bacteria. We believe the current work will set the stage for further development of detection, isolation, and characterization tools for new and existing strains of most common pathogenic Streptococci.

#179 - Uncovering Common RNA Functional Motifs Through Development of Novel RNA Structure Comparison and Clustering Approaches

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Ribonucleic acid (RNA) is a polymeric molecule that is fundamental to biological processes, with structure being more conserved than primary sequence. Advances in RNA structure characterization through experimental methods have led to an increase in the number of accurate secondary structures (SS) available, with over 50% of RNA-only structures in the Protein Data Bank arising since 2010. The past decade has also seen the rise of high-throughput structure probing assays that have generated experimentally supported structures for over 150 unique transcriptomes. As such, there remains the necessity to uncover common RNA structural motifs with a collective function through structural comparison and categorization. However, the challenge remains to develop fast and accurate RNA SS comparison and clustering algorithms that still capture fine detail in their structure representations. In this work, we present two approaches to this problem, built around an updated "structure array" sequence that assigns a single-character code to each nucleotide in the RNA, producing a flexible and economical structure representation. Our coarse-grained method uses cosine similarity of structure array k-mer count vectors, resulting in fast and efficient clustering of structures. We also present a fine-grained comparison approach that applies a customized global alignment method, utilizing an inverted context-specific affine gap penalty and a SS-specific substitution matrix. We evaluate our similarity scores in comparison to other methods by applying affinity propagation

clustering to a benchmark data set of known structure types. These scoring and clustering methods could be used to analyze high-throughput structure probing data sets to identify common structural motifs within the transcriptome, which could link RNA structure to function.

#205 - TARGET IDENTIFICATION OF CINACALCET TO INHIBIT VOLTAGE-GATED SODIUM CHANNELS

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Smith S. M., VA Portland

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Cinacalcet is a calcimimetic and an allosteric agonist of calcium sensing receptor (CaSR), decreases synthesis and secretion of parathyroid hormone by increasing CaSR sensitivity to calcium. It is FDA-approved for hyperparathyroidism. Recently, we found that modulators of the CaSR, including cinacalcet, were able to potently and indirectly inhibit voltage-gated sodium channels (VGSC) in neurons. VGSCs are proteins that mediate transmembrane sodium ion flow and thereby regulate membrane potential and neuronal communication. VGSCs are implicated in a variety of disease states including epilepsy, cardiac arrhythmias, and neuropathic pain. VGSC current blockage by cinacalcet was found to be mediated by G-protein coupled receptors (GPCR) in neurons. To identify the receptor(s) that mediate VGSC blockade by cinacalcet, we designed a chemoproteomics approach. A clickable photoreactive probe CP2 (cinacalcet probe 2) was designed and synthesized based on the structure of cinacalcet. It includes a photocrosslinker and clickable alkyne. CP2 retained VGSC inhibitory activity and was found to label proteins in mouse cortical neurons. Utilizing this CP2 probe, the labeled proteins are to be identified and validated to elucidate the unknown component(s) of this novel regulatory pathway.

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#173 - MOLECULAR PHARMACOLOGY OF P2X RECEPTOR LIGANDS VISUALIZED BY CRYO-EM

Oken A., I. Krishnamurthy, Lisi N., Godsey M. H., and Mansoor S. E., Oregon Health & Science University

Extracellular ATP is a critical signaling molecule that is found in a wide range of concentrations across cellular environments. The family of nonselective cation channels that recognize extracellular ATP, termed P2X receptors (P2XRs), is composed of seven sub-types (P2X1-P2X7) that assemble as functional homo-trimeric and hetero-trimeric ion channels. Each P2XR is activated at distinct concentrations of extracellular ATP, spanning from low nanomolar to millimolar, and expressed in an array of cell types. Sensing concentrations of ATP that diverge from homeostasis, P2XRs are implicated in a variety of pathophysiological diseases corresponding to where they are expressed and activated in the body. The therapeutic potential of P2XRs is an emerging area of research in which structural biology has provided avenues into potentially targetable sites on the receptor to prevent activation. In this regard, structure-based drug design can provide unique and insightful details into the intricacies of each receptor to facilitate sub-type selective ligands. Here, we highlight potential therapeutically targetable sites in the P2XR family and how single-particle cryogenic electron microscopy (cryo-EM) can be used to advance drug discovery, focusing on two cryo-EM structures; rP2X7 bound to ATP and rP2X7 bound to the noncompetitive antagonist JNJ-47965567.

#161 - IDENTIFICATION OF KDM5A-PHD1 DOMAIN BINDERS FOR ALLOSTERIC MODULATION OF THE EPIGENETIC DEMETHYLASE KDM5A

Ortiz G., Longbotham J., Zhang M. Y., Arkin M. R., and Galonic Fujimori D., UCSF

Epigenetic proteins post-translationally modify histone tails to regulate chromatin architecture and gene expression. The KDM5 family of histone demethylases act on histone 3 lysine 4 (H3K4) methyl marks to enable transcriptional repression. KDM5A acts as a regulator of development and differentiation, but its overexpression and mutagenesis can lead to lung, breast, colon, and leukemia cancers. Dysregulation of KDM5A also leads to

resistance to radiation and an increase in drug-resistant cancer cells. Inhibitors of KDM5A have been developed that bind to the catalytic site, but due to their competition with high cellular concentrations of the co-substrate 2-oxoglutarate required for demethylation, they have limited efficacy in vivo. KDM5A contains three plant homeodomain (PHD) that are involved in regulation of activity and scaffolding. The PHD3 domain recruits the demethylase to its substrate and the PHD1 domain allosterically stimulates the demethylation activity of KDM5A. PHD1 preferentially binds unmodified H3K4, the product of demethylation, through hydrogen bonding and electrostatic interactions between the protein and Lys4 residue in the histone H3 peptide. We envisioned targeting the regulatory role of the PHD1 domain to allosterically modulate KDM5A demethylation. A high throughput screen (HTS) was conducted to identify PHD1 binders that could potentially mimic binding of H3K4, serving as activators, or block H3K4 binding, serving as inhibitors. This presentation will outline the HTS campaign and development of a cellular proximity-based assay to identify potent and selective allosteric modulators of KDM5A. Chemical tools that target the PHD1 domain of KDM5A have the potential to treat KDM5A dysregulation in cells.

#157 - Cryo-EM Structure of Pre-Liganded NAIP5 Reveals Activation Mechanism of NAIP/NLRC4 Inflammasome

Paidimuddala B., Cao J., Xie Q., Wu H., and Zhang L., Oregon Health & Science University

Inflammasome is a cytosolic multiprotein complex formed in response to abnormal or pathogenic stimuli, and it initiates immune response to establish the innate immunity in mammals. Among the known inflammasomes, NAIP/NLRC4 inflammasome is specifically responsible for conferring immunity against various pathogenic bacteria. NAIP (nucleotide-binding domain, leucine rich repeat domain containing protein family (NLR family) apoptosis inhibitory protein) acts as a pathogen recognition receptor whereas NLRC4 (NLR family CARD containing protein 4) serves as a downstream molecule to undergo oligomerization to propagate and amplify the signal initiated by the activated NAIP. NAIP recognizes various bacterial ligands present in the cytosol of phagocytes, and the ligand bound NAIP further binds to NLRC4 and activates it by relieving its auto-inhibition. The activation of one NLRC4 molecule leads to exposure of its buried nucleation surface, which then serves as a binding site for another inactive NLRC4, to get activated. As a result of this process, a wheel-like NAIP/NLRC4 inflammasome composed of only one ligand bound NAIP and numerous NLRC4 molecules is formed. The CARD domain of NLRC4 in inflammasome interacts with CARD domain of pro-caspase-1 through CARD-CARD interactions and mediates the activation of pro-caspase-1. Caspase-1 activates the pro-inflammatory cytokines such as pro-IL-1β and pro-IL-18, which eventually induce the immune response that leads to pyroptosis (a form of programmed cell death) of the infected cell. Although, the information on overall inflammasome formation and its downstream signaling is known, no knowledge on how NAIP exits in inactive state in the absence of pathogenic ligand is available. The information on whether NAIP adopts the same auto-inhibited state as similar to NLRC4 or not, and how it is activated upon binding to ligand would provide valuable insights into the understanding of the activation of inflammasome formation and that further help design and develop the therapeutics for various inflammasome associated auto-immune diseases. Therefore, in this study, we determined the structure of pre-liganded mouse NAIP5 at the resolution of 3.3 Å by cryo-electron microscopy and found that it adopts an unprecedented wide-open conformation, with the nucleating surface fully exposed and accessible to recruit inactive NLRC4. Upon comparing it with the available liganded NAIP5 structures, we further found that the ligand binding could induces ~20° rotation of the winged helix domain (WHD) of NAIP5 and that triggers the conformational change of NLRC4 to propagate the inflammasome signal. Moreover, in our biochemical assays, we observed that the WHD loop of NAIP5 plays key roles in the inflammasome activation by relieving NLRC4 auto-inhibition, and stabilizing the formation of initial-encounter complex between liganded NAIP and active NLRC4. Overall, these data provide key insights into the understanding of the structural mechanisms of pre-liganded NAIP5, the process of NAIP5 activation, and the NAIP-dependent NLRC4 activation.

#131 - MULTI-OMICS APPROACHES TO DECIPHERING FORMALDEHYDE AND THE ONE-CARBON METABOLISM/SIGNALING INTERFACE

Pham V. N., Bruemmer K. J., and Chang C. J., University of California, Berkeley

One-carbon (1C) metabolism is a universal hub for cellular metabolism and epigenetic regulation. Consisting of the folate and methionine cycles, one-carbon metabolism maintains folate and S-adenosylmethionine (SAM) co-substrates as the primary one-carbon units for metabolic processes. We have identified formaldehyde—both an environmental genotoxin and a product of endogenous metabolism—as another key one-carbon unit. We have employed multi-omics approaches to identify and characterize protein targets of formaldehyde and downstream effects of formaldehyde on cellular methylation status, revealing novel mechanisms of formaldehyde in disrupting metabolic balance and one-carbon metabolism.

#180 - RNA INTERACTIONS WITH THE SARS-COV-2 N PROTEIN LINKER REGION

Stuwe H., Barbar E., Reardon P., Yu Z., **Shah S.**, and Hughes K., Oregon State University

The N protein of the SARS-CoV-2 virion is critical for viral genome packaging via RNA binding. The N protein can be divided into five main domains, and the central region is the linker, which is predicted to be disordered and has not been heavily studied. The linker is Serine-Arginine Rich, suggesting that it is a potential phosphorylation site. Phosphorylation is a critical process for the regulation of many cellular processes and can provide recognition sites for binding complexes. In a study that examined the recognition of the SARS-CoV-2 N protein by the human 14-3-3 protein, the linker was found to contain critical phosphosites for 14-3-3 binding. The goals of this project are to determine the structure, dynamics, and RNA interactions of the Serine-Arginine Rich linker region. To accomplish this, we performed Nuclear Magnetic Resonance (NMR) experiments to analyze the structure of the linker region of the N protein and its ability to bind viral RNA. NMR shows that the linker is not entirely unstructured and it is able to bind RNA. Other biophysical techniques such as Analytical Ultracentrifugation (AUC) and Multi-Angle Light Scattering (MALS) are used to identify the association state of the linker and the size of the resulting protein-RNA complex. We are currently working to biophysically characterize the structure, dynamics, and viral RNA binding ability of the R203M linker, which is present in the Omicron and Delta variants and thought to enhance viral infectivity. It is our directive to expand the study and investigate the effect of phosphorylation on linker-RNA interactions and the protein's dynamics using similar biophysical experiments.

#191 - CIRCRNA BARCODING ENABLES PRECISE QUANTIFICATION OF INTRACELLULAR PROTEIN-PROTEIN INTERACTIONS

Simon J., and Maly D., University of Washington

circRNA barcoding is a versatile, large-scale biochemical method that precisely quantifies changes in protein variant abundance. First, fusions of protein variants with an RNA binding protein are expressed in a mammalian environment where each mutant is tagged with a hyper-stable RNA barcode containing a unique identifying sequence. Subsequent enrichments of protein variants co-enrich the corresponding barcodes, allowing for protein quantification by sequencing. We demonstrate this method can be used in conjunction with proximity labeling to assess the intracellular protein-protein interactions of tens of thousands of variants with each of several binding partners. A unique benefit of this method is variant scoring is not impacted by differences in protein expression level, enabling higher confidence in loss of function scores than many deep mutational scanning or traditional 2-hybrid assays.

#195 - THIOL ACTIVATED 1,2,4-THIADIAZOLIDIN-3,5-DIONES RELEASE HYDROGEN SULFIDE THROUGH A CARBONYL SULFIDE DEPENDENT PATHWAY

Smith H. M., and Pluth M. D., University of Oregon

Carbonyl sulfide (COS) is a small gaseous molecule that has been detected previously in biological tissues and shares an intertwined history with the gasotransmitter hydrogen sulfide (H_2S). Carbonic anhydrase (CA) is a biologically ubiquitous enzyme that converts

COS to H_2S which is one of the reasons why COS is difficult to study endogenously. Prior work on 1,2,4-thiadiazolidin-3,5-diones (TDZNs) reported that these compounds release H_2S directly, albeit relatively inefficiently. Our mechanistic investigations, however, show that H_2S release from TDZNs is more complex than originally reported. The reaction of TDZNs with thiols produces common intermediates in the H_2S release pathway that are also present in known COS-based H_2S donors developed by our lab. Through different mechanistic investigations, we show that TDZNs efficiently release COS as the main pathway, which can then be converted to H_2S by CA. Additional mechanistic studies were performed to determine the role of the thiol-based nucleophile in the ring opening of TDZNs and also to investigate the impact of electronic substitution on COS/H_2S release. As a whole, this work demonstrates the usefulness of mechanistic investigations into competing release pathways for COS/H_2S donors and provides a readily modifiable donor scaffold for further application-based studies.

#207 - The Intercellular Heterogeneity of Raf Inhibition

Stoddard E., Fang L., Potter Z., Brush D., and Maly D., University of Washington

Raf is a family of serine/threonine kinases that are a key cog in the Ras-Raf-MEK-ERK MAPK pathway. The MAPK pathway is the mechanism by which cells convert extracellular stimuli, in the form of growth factors, to a transcriptional and post-translational output that regulates cell proliferation and differentiation. Mutations of MAPK pathway members often leads to increased flux through the pathway that often contributes to oncogenesis. Thus, developing inhibitors that target MAPK pathway members has been of high interest. Toward inhibiting Raf, a high degree of intercellular heterogeneity in Raf inhibitor potency is observed. We set out to understand the mechanisms behind this heterogeneity using LXH-254, a Raf inhibitor currently undergoing clinical trials, and LF-268, a novel Raf inhibitor developed within our lab. We observed a negative correlation between Raf activity and intracellular Raf inhibitor IC50s such that the more active Raf is (as measured by phosphor-MEK levels), the easier it is to inhibit. These IC50s were obtained by quantifying the effects of inhibitors on both downstream signaling and inhibitor-induced Raf dimerization. This correlation persists under perturbations of Raf activity including the inducible activation of Ras. We use a protein construct called Chemically Inducible Activator of Ras (CIAR) to induce Ras GTP-binding through a “switchable” guanine exchange factor (GEF). Upon activation of Ras, we observe decreased Raf inhibitor IC50s. The work described also offers insight into the mechanisms behind the synergy of MEK and Raf inhibition. It has been shown previously that some MEK inhibitors are synergistic with Raf inhibition toward decreasing proliferation of cancer cells and tumors. We demonstrate that only MEK inhibitors that induce Raf activity (as measured by pMEK) are synergistic with Raf inhibitors while those that do not are agnostic toward Raf inhibitor effects on downstream phosphorylation but antagonistic toward Raf inhibitor-induced dimerization. We believe this work informs key aspects of Raf inhibition that could lead to the development of more effective therapeutic strategies.

#135 - Detection of pathogenic E. coli by flow cytometry with an activity-based probe targeting mannose-mediated adhesion

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Adhesion is a critical pathogenic process mediated by the expression of adhesive proteins. The adhesion organelle, Type 1 pili, is expressed by most strains of *E. coli* and extends from the bacterial cell surface. Type 1 pili mediate bacterial binding to host cells through a mannose-sensitive binding event, facilitated by FimH, an adhesion protein located at the distal end of the Type 1 pilus that contains the mannose-binding site. FimH is therefore an attractive virulence factor to target for detection of pathogenic *E. coli*. Here, we report the development of a mannosylated activity-based probe (ABP) that strongly binds FimH at the mannose-binding site. Using tools from chemical proteomics and adhesion assays, we validated the efficacy of our ABP to target FimH in our model strain uropathogenic *E. coli* (UPEC). We then leveraged flow cytometric analyses to show that our FimH ABP binds a variety of pathogenic *E. coli* and can be used to enrich sorted populations for pathogens from a mixtures of bacteria in different matrices. We also observed strain-dependent probe labelling

efficiency and aim to use probe binding profiles coupled with machine learning to create a strain-specific detection method encompassing a variety of pathogenic *E. coli*. The ability of our FimH-specific ABP to distinguish variations in FimH expression presents high potential for the development of point-of-care diagnostic platforms to delineate infections caused by *E. coli*.

#189 - DEVELOPMENT OF NEW GENETIC TAGS FOR IMAGING CELLULAR PROTEINS

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Modern microscopy provides opportunities for viewing all aspects of cell biology. It is feasible to image multiple proteins at once by fluorescence microscopy (FM), super-resolution microscopy (SRM), or correlative light and electron microscopy (CLEM). These multi-scale studies require protein labels that are versatile: bright organic fluorophores for SRM, electron-dense nanoparticles for EM, and other novel reporters. With few options, most researchers rely on immunolabeling for SRM and EM, which has many drawbacks. The central goal of this project is to develop innovative and accessible strategies for analyzing cellular proteins across size scales and instrumentation. We developed Versatile Interacting Peptide (VIP) tags for this purpose. VIP tags consist of a heterodimeric coiled-coil between a genetically-encoded peptide tag and a reporter-conjugated “probe peptide”. Coiled-coils are a simple structural motif amenable to optimization by protein engineering. The dimerization specificity and affinity of VIP tags are dictated by the peptide sequence. To date, we have described three VIP tags: VIP Y/Z, VIPER, and miniVIPER. All three VIP tags enabled the selective labeling of a variety of cellular proteins and organelle structures. Importantly, this technology can be used to deliver a variety of biophysical reporters, including bright fluorophores (e.g., Cy5, BODIPY, xanthenes) and EM-optimized particles (e.g., gold or Qdots). VIP tags have been used in distinct applications, including pulse-chase labeling, observing endocytosis, and quantification of receptors in EM micrographs. For The Chemical Biology and Physiology Conference 21|22 we will present our progress developing two new genetic tags: TinyVIPER and PunyVIPER.

#187 - SUBCELLULAR POPULATION OF CB1 SIGNAL VIA DISTINCT G-PROTEINS

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In many cells, cannabinoid receptor 1 (CB1) is localized both at the cell surface (PMCB1) and on endomembranes (EMCB1). Both subpopulations have been suggested to exhibit different signaling functions. However, strategies for differentiating their relative contributions to cell signaling are scarce. We used HeLa cells as a heterologous expression system devoid of the endogenous receptor and employed amber stop codon suppression to incorporate a clickable amino acid in one of the extracellular loops of CB1. We employed genetically encoded biosensors to measure calcium ([Ca2+]i) and cAMP ([cAMP]i) levels in live HeLa cells. We showed that global CB1 stimulation led to an increase in intracellular [Ca2+]i while changes in intracellular cAMP levels differed between two subpopulations of cells with opposite responses. To dissect the two populations, we selectively labelled PMCB1 in live cells with a negatively charged impermeant tetrazine dye. The cells were then fixed and immuno-stained with a CB1 antibody allowing the identification of the EMCB1 subpopulation by detecting double versus single stained receptors. Single cell analysis permitted to dissect the relative functions of PMCB1 and EMCB1. We demonstrated a decrease of intracellular cAMP levels in cells with exclusive EMCB1 location, likely mediated by Gi/o coupling. In cells with partial PMCB1 location, activation increased intracellular cAMP levels via Gs coupling. This study highlights for the first time the differential roles of subcellular populations of CB1.

#123 - MOLECULAR DYNAMICS SIMULATIONS TO INVESTIGATE THE ROLE OF CURCUMIN IN ALZHEIMER’S DISEASE

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Alzheimer’s Disease (AD) is a neurodegenerative disease that affects 5.8 million Americans 65 years and older. Amyloid beta (Aβ), the primary component in AD plaques, is hypothesized to be toxic to cellular membranes where it interacts and nucleates fibril formation. It is proposed that Aβ’s toxicity is predominantly based on the amyloid cascade, where naturally occurring monomers

start to slowly form misfolded oligomers, that go on to form protofibrils. Protofibrils are small beta sheet rich structures that act as a seed to allow addition of more protofibrils until it leads to the formation of a mature fibrils. These mature fibrils are stabilized by interactions between hydrophobic amino acids. The oligomers are hypothesized to be the most toxic Aβ species. One hypothesized mechanism of toxicity is adhesion to the cell surface, which causes a disruption in the lipid membrane packing, leading to membrane thinning, stress on the membrane and ultimately a neurotropic effect in the synaptic cells. Curcumin is a polyphenolic compound found in turmeric that has demonstrated neuroprotection by membrane interactions that block Aβ binding. To investigate the chemical mechanisms of the hypothesized protection, molecular dynamics simulations were developed to model Aβ oligomer interactions with a model lipid membrane (DMPG). Corresponding simulation was developed with addition of curcumin to determine how the polyphenol alters the FO interactions with the membrane. The simulations were analyzed to measure the disturbance to the native membrane structure, Aβ oligomer structure, and determine the interactions between curcumin and Aβ. To determine the impacts of FO and curcumin on the membrane structure, the electron density contribution was calculated for the lipid tails, glycerol backbone, and headgroups. This data, in combination with calculated membrane thickness showed that the membrane was thinned in a localized region around the FO. When curcumin was introduced to the simulation, the thinned area decreased, but the magnitude of thinning was more drastic. The frequency of interactions between curcumin and Aβ was compared to the probability of random interaction based on the peptide's amino acid composition. Results indicate that curcumin preferentially binds to non-polar and cationic residues. These interactions influenced the structure of the membrane-bound FOs, measured through root mean square deviation (RMSD) calculations of the protein's backbone position. When curcumin was present, the FO's showed significant deviation from the starting structure compared to simulations performed without curcumin. In conclusion, it was determined through membrane thinning, amino acid interactions and RMSD that curcumin does have an effect on FO interactions with membranes. Overall, these initial results are preliminary data to investigate the molecular mechanism of curcumin as a potential therapeutic for AD through interactions with toxic Aβ oligomers.

#129 - DEVELOPMENT OF SMALL MOLECULES TO PROBE THE NUCLEAR LAMINA STRUCTURE AND FUNCTION IN LIVING CELLS

Warren J., Wang J., Dhoro F., Xiao X., and Li B., Oregon Health & Science University

The network of intermediate filament proteins that forms the nuclear lamina imparts rigidity and structural integrity to the mammalian cell nucleus. The major component proteins of the nuclear lamina include nuclear lamins (LA, LB1, LB2 and LC). They participate in essential nuclear functions that include mechanosensing, DNA repair, chromatin regulation, gene transcription and stem cell regulation. Mutations in the gene encoding for one of these lamin proteins, LA, have been implicated in a diverse set of diseases, ranging from skeletal and cardiac myopathies to lipodystrophies and progeria. These are collectively referred to as laminopathies. Changes to the size and shape of the nucleus are often observed in cells expressing these LA mutant proteins, suggesting that changes to the lamina structure and function contribute to the disease phenotypes associated with laminopathies. In order to understand the effect of mutant LA proteins on the nuclear lamina and its associated functions, it is essential to specifically investigate the lamina in an unaltered cell environment. Existing approaches to study the nuclear lamina are not appropriate for this purpose. We hypothesize that small molecule probes will be ideal. Previously, we identified a novel pyrolloquinazoline photoaffinity probe capable of selective nuclear lamin labelling. Here we seek to build on this work to develop a multifunctional photoaffinity probe that can readily undergo lamina labelling in live cells. A series of photoaffinity probe candidates have been synthesized and the primary target efficacy and specificity for the nuclear lamina has been evaluated. We report further development of this probe class as live cell labeling and imaging tools.

#181 - THE GCE4ALL RESEARCH CENTER: UNLEASHING THE POTENTIAL OF GENETIC CODE EXPANSION FOR BIOMEDICAL RESEARCH

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Genetic Code Expansion (GCE) is a very powerful, but also highly underutilized way to site-specifically place useful chemical groups into proteins. By creating engineered tRNA and aminoacyl-tRNA synthetase (tRNA/RS) pairs specific for non-canonical amino acids (ncAAs) and a repurposed codon, GCE uses the cell's ribosomal machinery to incorporate the ncAAs at genetically-encoded sites during protein synthesis. The ***GCE4ALL Research Center*** is a new research center funded by Oregon State University and the Biomedical Technology Development and Dissemination program of the National Institute of General Medical Sciences. The *GCE4All Research Center* is dedicated to the task of improving GCE tools so that biomedical researchers around the world can easily adopt and use GCE technologies in their own labs to generate specially designed forms of proteins for probing and visualizing how life works.
The *GCE4All Research Center* mission is to improve and disseminate this powerful set of technologies so they can be more widely used for revealing mechanisms of health and disease and developing improved diagnostics and therapeutics.
Research efforts in the *Center* are organized in two **Technology Development Projects** focused, respectively, on making optimized GCE tools to incorporate ncAAs relevant for:
Bioorthogonal ligations; and
Biochemical probes and post-translational modifications.
The *Center* will "road test" developing GCE technologies through formal collaborations with selected researchers in the context of authentic, challenging problems in biomedical research – referred to as **Driving Biomedical Projects**.

Our presentation here will provide an overview of the *GCE4All Research Center* structure, describe our approaches to technology development and dissemination, and introduce the initial set of technologies that we have targeted for improvement and wide adoption.
Also, as you have interest, please:
Explore our resources at
https://gce4all.oregonstate.edu/
Contact us at gce4all-center@oregonstate.edu

#159 - A NEAR-INFRARED FLUORESCENT PROBE FOR IMAGING OF Cu2+ IN HUMAN BRAIN TISSUE

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Copper is an essential trace metal and participates in various physiological pathways and biological processes, including energy metabolism, neuromodulation and antioxidative defense. Copper homeostasis is of vital significance to living organisms. Imbalanced brain copper content has long been associated with neurodegenerative diseases, such as Wilson's disease, Menkes disease, Alzheimer's disease and Parkinson's disease. In Parkinson's disease, studies have revealed that the copper levels in the substantia nigra and locus coeruleus are decreased by 55% compared with those in healthy brains. The copper contents in the brains of Alzheimer's disease patients are highly heterogenous, showing elevated copper in amyloid plaques, and up to 50% decrease of copper levels in the hippocampus, frontal cortex, cerebral cortex and amygdala. In healthy brains, the distribution of copper is heterogeneous. Locus coeruleus and substantia nigra were revealed to exhibit the highest copper levels. Disorders in copper levels can not only lead to neurodegenerative diseases, but have also been demonstrated to be correlated to the progression of neuroblastoma. Neuroblastoma is the third most common cancer in the childhood. It has been reported that copper levels in neuroblastoma cell lines are 50% higher than normal human fibroblasts. Homeostasis of copper in living organisms is essential for health: both deficiency of copper and excess of copper lead to disease.
Fluorescent probes are an effective tool for visualization of biological species in living systems using fluorescence imaging technique. We have developed a near-infrared fluorescent probe **CyCu1** for Cu^{2+}. In response

to Cu^{2+}, **CyCu1** undergoes a change in fluorescence that can be harnessed by confocal microscopy, as well as a change in absorption that can be harnessed by photoacoustic imaging. The probe showed good stability in aqueous solution and exhibited excellent selectivity for Cu^{2+} over other metals. Preliminary fluorescence and photoacoustic imaging have demonstrated that **CyCu1** can be used in applications of fluorescence/photoacoustic dual-modality imaging of Cu^{2+}. **CyCu1** was then applied to the imaging of Cu^{2+} in biological models using confocal microscopy. **CyCu1** not only showed capability to detect Cu^{2+} in tumor tissue, but can also distinguish human brain tissue from different regions. **CyCu1** has been demonstrated as a promising tool for the study of Cu^{2+} in tissue samples.

#176 - PHOSPHOTRIESTERASE BASED CHEMICAL-GENETIC TOOLS FOR CONTROLLED RELEASE OF CHEMICAL REPORTERS

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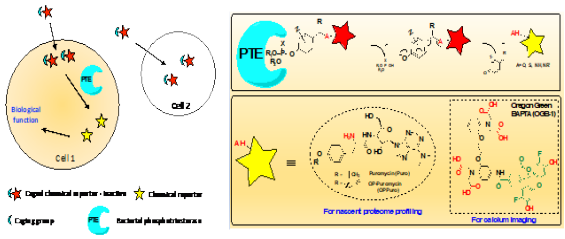
The spatial targeting of chemical reporters like drugs, fluorescent probes and sensors is of much utility, and there is still a need for robust systems. Genetically encoded sensors (protein based, for calcium, voltage sensing etc.) have their limitations. Chemical-genetic hybrids are promising, as they combine genetically encodable proteins with the robust performance of chemical reporters. One such hybrid system is caging-uncaging. The desired chemical reporter is caged, thus non-reactive, and is uncaged exclusively by a genetically encoded uncaging enzyme. However, existing uncaging systems have some limitations (slow kinetics, limited modulation etc.).
Here we present a new, robust orthogonal caging-uncaging system based on phosphotriester-triesterase chemistry for cell specific delivery of chemical reporters like; (1) puromycin that covalently attaches to nascently synthesized polypeptides, for nascent proteome profiling; (2) chemical calcium sensor Oregon Green BAPTA-1, for cell specific calcium imaging. Phosphotriesters are xenobiotic, and hence stable unless uncaged by a phosphotriesterase (PTE), thus making the system orthogonal. Phosphotriesterase (PTE) is of bacterial origin, is highly catalytically efficient, and is highly engineerable allowing for tailoring as per need. We demonstrated this system by caging the reactive amino group of puromycin, and the carboxylates of the calcium sensor Oregon Green BAPTA-1 (OGB-1). We thus made them non-responsive, and then selectively uncaged in PTE expressing cells, thus allowing spatial control.



#112 - DESIGN OF LOV2 CIRCULAR PERMUTANTS FOR OPTOGENETIC CONTROL OF CALCIUM SIGNALS AND CELLULAR PHYSIOLOGY

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Zhou Y., Texas A&M University

Plant-based photosensors, such as the light-oxygen-voltage sensing domain 2 (LOV2) derived from Avena sativa phototropin 1, can be modularly wired into the cell signaling network to remotely control protein activity and physiological processes. However, given that effector domains can only be fused to the C-terminal Jα helix, the wide applicability of LOV2 is hampered by the limited choice of available caging surfaces. Here, we engineered a set of LOV2 (cpLOV2) with additional caging capabilities, thereby expanding the repertoire of genetically-encoded photoswitches to accelerate the design of optogenetic devices. By fusing cpLOV2 with calcium channel-activating fragments derived from stromal interaction molecule 1 (STIM1), which are light-operated calcium channel actuators (LOCCa), we can remotely control calcium influx and oscillation. The cpLOV2 can also be used as a modular light-sensitive allosteric switch to control STIM1 activity and achieve photo-inducible calcium ON or OFF-switch.

Besides, we demonstrate the use of cpLOV2-based optogenetic tools to antagonize CRISPR/Cas9-mediated genome engineering, control protein subcellular localization, reprogram transcriptional outputs, and generate photoactivatable CAR T-cells for inducible tumor cell killing. Our approach is widely applicable for engineering other photoreceptors to meet the growing need of optogenetic tools tailored for biomedical and biotechnological applications.

#153 - Optogenetic Control of Non-Apoptotic Cell Death

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Herein, a set of optogenetic tools (designated LiPOP) that enable photoswitchable necroptosis and pyroptosis in live cells with varying kinetics, is introduced. The LiPOP tools allow reconstruction of the key molecular steps involved in these two non-apoptotic cell death pathways by harnessing the power of light. Further, the use of LiPOPs coupled with upconversion nanoparticles or bioluminescence is demonstrated to achieve wireless optogenetic or chemo-optogenetic killing of cancer cells in multiple mouse tumor models. LiPOPs can trigger necroptotic and pyroptotic cell death in cultured prokaryotic or eukaryotic cells and in living animals, and set the stage for studying the role of non-apoptotic cell death pathways during microbial infection and anti-tumor immunity.

#139 - Developing a library of non-canonical substrates for mining amidase activity

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Amide hydrolysis is associated with a wide range of important enzymes (proteases, hydrolases, carboxypeptidases etc.) that are relevant to nutrient cycling, nitrogen access from complex plant biomass, and waste recycling, all within the purview of the U.S. Department of Energy. We therefore are interested in experimentally “mining” for amidases that have unknown, and potentially useful, degradative activity. To facilitate the identification of amidases and profile amidase activity, a diverse library of 130 non-canonical fluorogenic amidase substrates were synthesized. A fluorescence-based assay was used to screen library compounds against biological samples and rapidly detect active substrates. Potential substrates identified by the screen were used to guide the development of activity-based probes (ABP) capable of selectively labeling active enzymes and enable subsequent proteomic characterization. Here, we report the successful deployment of this approach using the yeast strain, *Yarrowia lipolytica*, as a model system. Twenty-two potential substrates were identified during initial library screenings. Two of these substrates were selected for ABP development. SDS-PAGE and fluorescence gel imaging were used to evaluate the potency and specificity of the ABPs. We hope to use this approach to understand the full scope of proteins that microorganisms employ for nitrogen acquisition in response to environmental changes, and that discovery of amide hydrolases with unique substrate specificity profiles will be useful for future bioproduct formation applications.

#168 - Chemical biology approaches to elucidating plant stress response and plant-microbe interactions in Sorghum bicolor

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Sorghum bicolor is a grass species grown worldwide for its grain, making it the 5th most

important cereal crop and a key plant species for bioenergy production. Understanding sorghum's response to environmental stressors such as drought and identifying beneficial plant-microbe interactions are essential to more robust and sustainable cultivation of sorghum in a changing climate. Engineered microbes can be strategically deployed to promote crop health and productivity. However, biocontainment strategies that precisely control the environmental niche of introduced microbes may be required for safe application. To investigate our capacity to control the environmental persistence of an engineered microbe, we aim to establish metabolic addiction of bacteria to plant root exudate compounds, specifically focusing on identifying bacteria capable of using sorgoleone, a hydrophobic sorghum secondary metabolite, as a sole carbon source for growth. Here we report three bacteria capable of utilizing sorgoleone as a sole carbon source: a Burkholderia, Pseudomonas, and Acinetobacter species. Sorgoleone was extracted from the roots of sorghum seedlings, purified, and subsequently used to prepare synthetic sorgoleone media for bacterial culture. We used differential transcriptomics of these strains grown on sorgoleone and acetate to identify genes that are likely associated with sorgoleone utilization. To investigate the proteins that may be involved in sorgoleone catabolism and enable a more global search for microbes that interact with sorgoleone, a photoaffinity probe based on the structure of sorgoleone was synthesized and applied to bacteria cultured on sorgoleone. SDS-PAGE revealed distinct protein profiles for bacteria grown on sorgoleone compared to acetate, and proteomics analysis to identify key proteins is now underway. More broadly, we have applied activity-based probes to characterize sorghum responses to drought. Plants under water stress use proteases to degrade proteins and recycle key building blocks for synthesis of essential drought response proteins and metabolites; however, excessive proteolytic activity leads to irreversible tissue damage and loss of function, and thus protease activity must be carefully regulated in different tissues. In vivo activity-based protein profiling of sorghum roots revealed an overall decrease in root serine and cysteine protease activities that may preserve critical root functions under severe water limitation. These chemical biology approaches will provide important complementary data to ongoing transcriptomic and metabolomic studies that will clarify the complex interactions between sorghum, the soil microbiome, and the environment.

#146 - Optogenetic Reconstruction of Store-Operated Calcium Channels

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Store-operated Ca^{2+} entry mediated by STIM1 and ORAI1 constitutes one of the major Ca^{2+} entry routes in mammalian cells. Physiologically, the molecular choreography of STIM1–ORAI1 coupling is initiated by endoplasmic reticulum (ER) Ca^{2+} store depletion with subsequent oligomerization of the STIM1 ER-luminal domain, followed by its redistribution towards the plasma membrane to gate ORAI1 channels. Over the past five years, we have engineered a series of genetically-encoded Ca^{2+} actuators (GECAs) derived from STIM and ORAI to enable optical control of Ca^{2+} signaling with unprecedented spatial and temporal resolution. STIM1 is an ER Ca^{2+} sensor that oligomerizes and conformational switch in response to store depletion, overcoming autoinhibition and forming clusters that then couples to ORAI channel. Two general engineering approaches have been applied to confer photosensitivity to STIM1: i) fusion of a photosensory module with SOAR/CAD to recapitulate reversible CC1-SOAR/CAD intramolecular trapping (Opto-CRAC-V1); ii) replacing the STIM1 luminal domain and the transmembrane domain with a light-inducible oligomerization domain (Opto-CRAC-V2). These tools enable light-dependent STIM-ORAI coupling to elicit Ca^{2+} influx through highly Ca^{2+}-selective CRAC channels in both excitable and non-excitable cells.

Meanwhile, we designed a light-operated Ca^{2+} channel (designated LOCa) by inserting a plant-derived photosensory module into the intracellular loop of an engineered ORAI1 channel. LOCa displays biophysical features reminiscent of the ORAI1 channel, which enables precise optical control over Ca^{2+} signals and hallmark Ca^{2+}-dependent physiological responses. Compared to traditional pharmacological or genetic tools, GECAs rival by high precision, rapid reversibility, facile tunability and non-invasiveness. These GECAs offer new non-invasive means to probe the structure-function relations of calcium channels and significant

advances toward the use of optogenetics for immunomodulation and neuromodulation.

#104 - MOLECULARLY ENGINEERED SURFACTIN BIOSURFACTANT TO TREAT MDA-MB-231, TRIPLE NEGATIVE BREAST CANCER

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Biosurfactants are amphiphilic molecules that possess biological properties such as anti-cancer. Their synthesis by bacterial fermentation can yield large quantities, creating opportunities for the development of low-cost therapeutics. Furthermore, microbial biosurfactant synthesis provides safe "green" routes to low toxicity natural products. Research presented focuses on the natural anti-cancer properties of surfactin, a lipopeptide biosurfactant. The functionality of this molecule enables its use as a scaffold for molecular engineering of its structure to interrogate structure-property relationships to achieve optimum efficacy and minimal toxicity.

Surfactin is a negatively charged amphiphile composed of a cyclized heptapeptide (L)-Glu-(L)-Leu-(L)-Leu-(L)-Val-(L)-Asp-(D)-Leu-(L)-Leu, and 11-15C β-hydroxy fatty acid that forms an amide with the α-amino group of (L)-Glu and an ester group with the α-carboxyl moiety of (L)-Leu. There are multiple regions of interest for modification, including the glutamic and aspartic acid residues as well as the lactone in the peptide ring that can be ring-opened by amine nucleophiles. Modifications thus far focus on conjugating amine functional moieties to glutamate and aspartate that: i) amplify the negative charge, ii) introduce positive charge and iii) present neutral polar entities. By molecular engineering of surfactin analogs, we hope to discover structures with improved anti-cancer activity and reduced toxicity.

The chosen cell line for this work is the MDA-MB-231, triple negative breast cancer cell (TNBC) line. TNBCs account for 15% of all breast cancers, and are negative for estrogen, progesterone, and HER2 hormonal receptors – thus there are few treatment options. Toxicity studies on healthy breast derived fibroblasts and erythrocytes were used to determine therapeutic indices. Preliminary research indicates that cationic derivatives have the highest cytotoxicity against MDA-MB-231 cells, while anionic compounds prove to have the safest therapeutic indices. Critical Micelle Concentrations indicate that molecularly engineered surfactins have lower CMCs than that of the natural component.

The hypothesis of this research is that molecularly engineering surfactin will be identified with a high selectivity index against MDA-MB-231 breast cancer relative to normal cell lines. Hypothesis is supported with the use of molecularly engineered biosurfactants, *in vitro* cell toxicity analysis, and further characterization of biosurfactants utilized. Upon the conclusion of the proposed project, insight into the synthesis, modification, and characterization surfactin analogues will provide information on lead compounds for anti-cancer applications. Subsequent *in vitro* assays of molecularly engineered surfactin on MDA-MB-231 will provide structure-activity relationships to determine cytotoxicity and mechanism of action. Further characterization will allow for future studies of glycolipid and lipopeptide modified biosurfactants for medicinal applications.

#192 - BRIGHT FLUORESCENT CONJUGATES FOR IMAGING APPLICATIONS WITH ERASABLE SIGNAL VIA DUAL-RELEASE MECHANISM

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Cell analysis techniques like flow cytometry and fluorescence microscopy are widely used to explore cell biology and provide important insights into a variety of physiological and pathological processes. These techniques require bright and specific staining reagents to enable reliable read-out, making the choice of fluorescent labels a key aspect of experimental design. Conventional labels, however, usually do not permit the high multiplexing and multiparameter analysis that is often required for samples with high diversity. This is mainly due to the limitations of the spectrum detection range used in fluorescence-based applications, which in turn limits the depth of phenotypic analysis to a small number of expression markers. In order to address this limitation, several approaches of releasable labels have been developed. In particular, REAlease® and REAdyelease® conjugates were designed for this purpose, thus enabling subsequent staining and imaging. Ultimately, it

was demonstrated on the MACSima™ Cyclic Imaging Platform that these reagents permit cell analysis with a potentially unlimited number of parameters, allowing the identification of potential targets for immune therapy against solid tumors. However, a challenge exists when fluorescent conjugates are not completely released from the tissue section, resulting in carryover signal from previous imaging cycles and ultimately a lower depth of analysis. Here it becomes evident that the number of imaging cycles is not only determined by the number of available conjugates but also by their release efficiency. In this work, we present a promising approach for the design of the aforementioned conjugates with the aim to further increase the efficiency of dye release without compromising their brightness. This approach relies on conjugates decorated with fluorophores via two spacers (polymer backbones) that can orthogonally be digested by two different enzymes, thus permitting efficient release of the dyes from the binder. In addition, such design allows high multimerization of the fluorophores leading to the increased brightness of the probes that is especially helpful for reliable signal detection, e.g. in the analysis of rare epitopes.

#103 - IONIC COMPLEXED NANOPARTICLES FOR HEPARIN DRUG DELIVERY

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Patient compliance, where the patient follows a prescribed and dispensed regimen as per the prescriber's order, is an important aspect of promoting wellness in patients. There are problems that arise with current heparin delivery methods which lead to low patient compliance. The goal of this research is to develop a more robust method of heparin delivery so that the problem of patient compliance is mitigated.

Heparin, a highly sulfated glycosaminoglycan that functions as an anticoagulant drug to prevent pulmonary embolisms, deep vein thrombosis, and peripheral arterial embolism, which are common post-surgical complications. It is injected subcutaneously as either inpatient or outpatient. Due to pain associated with injection, and expensive in-patient fees, patients are less likely to comply with the requirements of heparin administration. This project aims to increase patient compliance by creating a targeted approach for heparin oral drug delivery. Oral drug delivery is one of the safest and painless forms of drug administration by patients.

Heparin is a negatively charged drug due to its carboxyl and sulfate groups. It is a heterogeneous mixture containing sulfated monosaccharide residues of L-iduronic acid and D-glucosamine that are 1→4 linked. Therefore, it is an excellent candidate for delivery by incorporation in ionic complexed nanoparticles. These nanoparticles consist of a cationic polymer entrapping an anionic drug. This method of creating nanoparticles has been used to load charged therapeutics such as DNA, drugs, or probes for a variety of biomedical applications.

The cationic polymer being used in this research project is chitosan. It is a naturally occurring amino polysaccharide with repeat units of D-glucosamine linked β-(1→4). Chitosan is produced from the deacetylation of chitin obtained from crustaceans. The polymer is cationic due to the amino groups present. Chitosan has been introduced to the pharmaceutical industry as tablet binders, coating materials and for gene and peptide delivery. Chitosan is biodegradable and nontoxic, making it suitable for biomedical applications. It is beneficial for drug delivery because it is mucoadhesive, meaning it adheres to the mucosal epithelial layer of the GI tract. This results in enhanced penetration of large molecules, such as heparin, through the epithelial layer.

In this research we are developing ionic complexed nanoparticles (ICNs) with chitosan to encapsulate heparin for use in oral drug delivery. ICNs are pH sensitive such that they can be used to release drugs based on cue within specific pH ranges. We are using this pH sensitivity by designing the heparin bound ICN's to be stable under the acidic conditions (pH 1.5-2.0) within the stomach and for a short time in the small intestines where it will pass through the epithelial layer and release the drug at pH values about 7.4. In other words, the drug containing ICNs must pass through and survive the conditions of the GI tract before reaching the small intestines. Due to its large size, highly negative charge and high-water solubility, administration of heparin

without a drug carrier results in poor absorption within Gastrointestinal (GI) tract. To ensure that the designed ICN heparin delivery systems can meet the strict requirements for oral drug delivery, we are studying ICN particle stability and heparin release as a function of time, pH, temperatures using simulated fluids that mimic the GI tract environment. Variables in the design of ICNs include molecular weight of both heparin and chitosan as well as addition of surfactants and emulsifiers to further stabilize the ICNs. Successful completion of this work will result in efficient heparin or formulations.

#126 - CAFFEINE-OPERATED SYNTHETIC MODULES FOR CHEMOGENETIC CONTROL OF PROTEIN ACTIVITIES BY LIFE STYLE

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Here we introduce a robust chemically-induced dimerization (CID) system. that is genetically-encoded caffeine-operated synthetic module (COSMO). With commercially available caffeine or caffeine-containing energy drinks, like starbucks, Ccoco cola or redbull. this tool can be applied to control calcium entry, activate FGFR-mediated cell signaling, boost the binding of nanobodies against the RBD domain from the SARS-CoV-2 spike protein, COSMO can also be used to kill cancer cells and enable rapid actin cytoskeleton remodeling. This system is evolved from an anti-caffeine nanobody through high-throughput screening. We further make it among the most potent CID systems like the FKBP/FRB/rapamycin system after connecting two copies of COSMO with a shorter linker. And when two copies of COSMO are connected with a longer linker, it will favor intramolecular dimerization. When these two COSMO are inserted in host proteins, they will achieve tailored function. As caffeine is cheap and readily available in daily drinks, these chemical biology tools will greatly reduce the cost in engineering proteins or cells of therapeutic potentials.