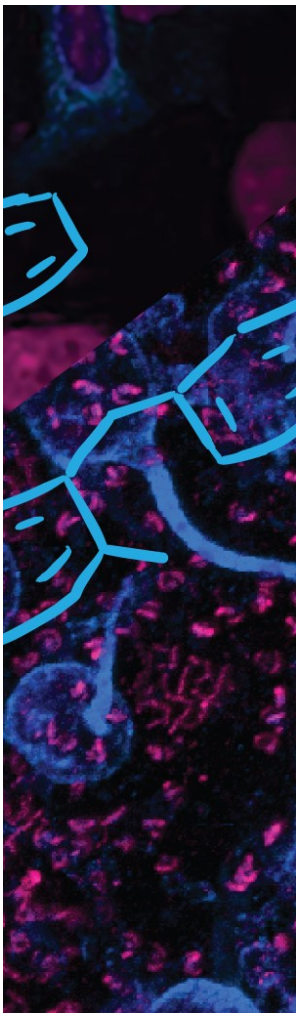
Program and Abstracts

December 12-15, 2019
Oregon Health & Science University
Portland, OR USA

OHSU



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KNIGHT CANCER Institute

Chemical Science

ROYAL SOCIETY OF CHEMISTRY

IMPACT FACTOR 9.556*

Organic & Biomolecular Chemistry

ROYAL SOCIETY OF CHEMISTRY

IMPACT FACTOR 3.490*

RSC Advances

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CHEMBIOCHEM
SYNTHETIC BIOLOGY & BIO-NANOTECHNOLOGY
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CONFERENCE ORGANIZING COMMITTEE:
Michael Cohen, Ph.D.
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Xiangshu Xiao, Ph.D.

SESSION CHAIRS:
Veronica Cochran, Ph.D. Student
Skylar Ferrara, Ph.D.
Julia Huey, Ph.D. Student
Erika Riederer, Ph.D. Student
Kelsie Rodriguez, Ph.D. Student
Jon Savage, Ph.D. Student
Carsten Schultz, Ph.D.
Francis Valiyaveetil, Ph.D.

EVENT PLANNERS:
Amy Johnson - email: johnsamy@ohsu.edu
Alanna Lapp - email: lapp@ohsu.edu
Elie Inns - email: inns@ohsu.edu

SPECIAL THANKS TO:
Tim McCormick for AV support

All activities take place at the:

Knight Cancer Research Building
1st Floor
Oregon Health & Science University
2720 S.W. Moody Avenue
Portland, Oregon 97201 USA

THURSDAY – DECEMBER 12, 2019	
11:00 – 13:00	Registration and Welcome
13:00 – 13:10	Opening Remarks
SESSION 1: CHEMICAL PHYSIOLOGY Chair: Skylar Ferrara	
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13:45 – 14:20	**Martin Kelly**, Oregon Health & Science University, USA *Elucidating Hypothalamic Circuits Controlling Homeostatic Functions Using Optogenetics*
14:20 – 14:35	Short Talk: **Anupam Patgiri**, Harvard Medical School, USA *An Engineered Enzyme to Alleviate Reductive Stress in Mitochondrial Disease*
14:35 – 15:05	Break
15:05 – 15:40	**Raymond Moellering**, University of Chicago, USA *Chemical Proteomic Platforms to Expose and Exploit Novel Metabolic Signals in Disease*
15:40 – 16:15	**Michael Fischbach**, Stanford University, USA *Small molecules from the human microbiota*
16:15 – 16:30	Short Talk: **Erin Carlson**, University of Minnesota, Twin Cities, USA *Chronic exposure to complex metal oxide nanoparticles elicits rapid bacterial resistance*
16:30 – 17:10	Flash Talks: Poster presenters (1.5 min/1 slide)
17:10 – 17:20	Break
17:20 – 18:20	KEYNOTE SPEAKER – Chair: Skylar Ferrara **Benjamin Cravatt**, The Scripps Research Institute, USA *Activity-based proteomics – protein and ligand discovery on a global scale*
18:20 – 20:00	Conference Dinner and Reception

FRIDAY – DECEMBER 13	
SESSION 2: IMAGING Chair: Jon Savage	
09:00 – 09:35	**Amy Palmer**, University of Colorado, Boulder, USA *Riboglow: a new platform for tracking RNA molecules in live mammalian cells*
09:35 – 10:10	**Kazuya Kikuchi**, Osaka University, Japan *New Biological Findings which were Revealed by Designed Fluorescent Probes*
10:10 – 10:25	Short Talk: **Alison Tebo**, Sorbonne Université, France *Split fluorescent reporters with rapid and reversible complementation*

10:25 – 10:55	Break
10:55 – 11:30	**Luke Lavis**, HHMI Janelia, USA *From single-molecule imaging to the brain: A circuitous route to new neural activity indicators*
11:30 – 12:05	**Peter Wang**, University of California, San Diego, USA *Live Cell Imaging and Cellular Reprogramming in Immuno-engineering*
12:05 – 12:20	Short Talk: **Rahul Singh Kathayat**, The University of Chicago, USA *Chemical approaches to probe the regulation of S-palmitoylation*
12:20 – 13:00	Flash Talks: Poster presenters (1.5 min/1 slide)
13:00 – 14:00	Lunch Meet the Speakers
14:00 – 15:30	Poster Session I (odd poster numbers)
SESSION 3: OPTICAL TOOLS Chair: Carsten Schultz	
15:30 – 16:05	**David M. Chenoweth**, University of Pennsylvania, USA *Design and synthesis of new chemical tools for probing, manipulating, and imaging biological systems*
16:05 – 16:40	**Graham Ellis-Davies**, Mount Sinai, USA *Physiology with caged compounds*
16:40 – 17:15	**James Frank**, Oregon Health & Science University, USA *Optical tools for manipulating classical and orphan cannabinoid receptors*
17:15 – 18:15	KEYNOTE SPEAKER – Chair: Julia Huey **Eric Gouaux**, Oregon Health & Science University, USA *Architectures and mechanisms of signaling at the chemical synapses of the brain*

SATURDAY – DECEMBER 14

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09:35 – 10:10	**Matthew Simon**, Yale University, USA *Chemical tools to study the dynamics of the transcriptome*
10:10 – 10:25	Short Talk: **Linglan Fang**, University of Washington, USA *A chemoproteomic platform for profiling the cellular interactome of protein kinases*
10:25 – 10:55	Break
10:55 – 11:30	**Andreas Marx**, University of Konstanz, Germany *Friends or Foes? News on old Nucleotides*
11:30 – 12:05	**Jennifer Heemstra**, Emory University, USA *A chemical biology toolbox for RNA post-transcriptional modification and capture*
12:05 – 12:20	Short Talk: **Elisar Barbar**, Oregon State University, USA *The LC8-RavP ensemble structure evinces a role for LC8 in regulating Lyssavirus polymerase functionality*

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13:00 – 14:00	Lunch Meet the Editors
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15:30 – 16:05	**Jennifer Kohler**, UT Southwestern Medical Center, USA *Intestinal fucose, in sickness and in health*
16:05 – 16:40	**Linda Hsieh-Wilson**, California Institute of Technology, USA *Neuroplasticity and the Sweeter Side of Cell Signaling*
16:40 – 16:55	Short Talk: **Markus Lakemeyer**, Stanford University, USA *Profiling of proteases from commensal bacteria that modulate protease-activated receptor (PAR) signaling*
16:55 – 17:55	KEYNOTE SPEAKER – Chair: Veronica Cochrane **Laura Kiessling**, Massachusetts Institute of Technology, USA *Glycans as Microbial IDs*

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SESSION 6: PROTEIN MAGIC Chair: Erika Riederer	
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09:05 – 09:40	**Matthew Pratt**, University of Southern California, USA *O-GlcNAc as a multifaceted inhibitor of amyloid aggregation*
09:40 – 09:55	Short Talk: **Leah Frye**, Schrödinger *Computationally-Driven, Structure-Based Drug Discovery*
09:55 – 10:25	Break
10:25 – 11:00	**Hermen Overkleeft**, Leiden University, The Netherlands *Activity-based glycosidase profiling in biomedicine and biotechnology*
11:00 – 11:35	**Virginia Cornish**, Columbia University, USA *Expanding the Synthetic Capabilities of Yeast*
11:35 – 12:35	KEYNOTE SPEAKER – Chair: Francis Valiyaveetil **Tom Muir**, Princeton University, USA *Painting Chromatin with Synthetic Protein Chemistry*
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12:45	Lunch

DESIGN AND SYNTHESIS OF NEW CHEMICAL TOOLS FOR PROBING, MANIPULATING, AND IMAGING BIOLOGICAL SYSTEMS

Dr. David Chenoweth - University of Pennsylvania

Chemical tools are invaluable for modulating, probing, manipulating, and imaging biological systems. Our laboratory utilizes synthetic chemistry to develop new small molecule and peptide based chemical tools for probing and monitoring biological systems in a spatially defined and temporally controlled manner. Recent results from our laboratory describing modular chemical tools for control of protein localization have paved the way for several recent advances aimed at controlling cellular processes. This work will be discussed in the context of new chemical tools for studying biology.

EXPANDING THE SYNTHETIC CAPABILITIES OF YEAST

Dr. Virginia Cornish - Columbia University, New York, New York

In vitro directed evolution allows biomolecules with new and useful properties to be engineered—mimicking natural evolution on an experimentally accessible time scale by creating large libraries of DNA mutants using PCR and then carrying out a high-throughput assay for variants with improved function. To provide a breakthrough in the complexity of libraries that can be readily searched experimentally for synthetic biology and to allow systems to be directly engineered in the cell, my laboratory is engineering S. cerevisiae so that both the mutagenesis and selection steps of directed evolution can be carried out entirely in vivo, under conditions of sexual reproduction. We have built a modular chemical complementation assay, which provides a selection for diverse chemistry beyond that natural to the cell using themes and variations on the yeast two-hybrid assay. In addition, we devised a heritable recombination system, for simultaneous mutagenesis and selection in vivo under conditions of sexual reproduction. Finally, we have begun to utilize these mutagenesis and selection technologies to engineer yeast to carry out new functions themselves ranging from being a biosensor, to a therapeutic, to a self-organizing community.

ACTIVITY-BASED PROTEOMICS – PROTEIN AND LIGAND DISCOVERY ON A GLOBAL SCALE

Dr. Benjamin Cravatt - Department of Chemistry, The Scripps Research Institute

Advances in DNA sequencing have radically accelerated our understanding of the genetic basis of human disease. However, many of human genes encode proteins that remain uncharacterized and lack selective small-molecule probes. The functional annotation of these proteins should enrich our knowledge of the biochemical pathways that support human physiology and disease, as well as lead to the discovery of new therapeutic targets. To address these problems, we have introduced chemical proteomic technologies that globally profile the functional state of proteins in native biological systems. Prominent among these methods is activity-based protein profiling (ABPP), which utilizes chemical probes to map the activity state of large numbers of proteins in parallel. In this lecture, I will describe the application of ABPP to discover and functionally annotate proteins in mammalian physiology and disease. I will also discuss the generation and implementation of advanced ABPP platforms for proteome-wide ligand discovery and how the integration of these global 'ligandability' maps with emergent human genetic information and phenotypic screening in

model organisms can expand the druggable fraction of the human proteome for basic and translational research objectives.

TRANSLATING RNA SEQUENCE INTO LEAD SMALL MOLECULE MEDICINES

Prof. Matthew Disney - Department of Chemistry, The Scripps Research Institute

About 80% of our genome is transcribed into RNA and only about 2% is translated into protein. Yet, drug discovery focuses almost exclusively on targeting protein. A major challenge in Medical Science has been exploiting targets for drug development. Our programmatic is on developing technologies to decipher which cellular RNAs are “druggable” targets for small molecules and which small molecules can target them. Here, we will describe advances in the area of Small Molecules Interacting with RNA (SMIRNAs), including a sequence-based small molecule rational design tool dubbed Inforna. It has enabled the design of SMIRNAs against RNAs that cause hard to treat cancers and incurable genetically defined disease that have no known treatment by scanning for druggable pockets across human RNA sequence. We will describe these compounds and their implications advancing lead medicines and also as chemical probes to understand previously unknown RNA biology. We will also describe the development of approaches that allow for targeted degradation of RNAs in cells and animals by using SMIRNAs. For example, we can recruit endogenous cellular nuclease to cleave RNAs selectively and sub-stoichiometrically in cells and animals. There is great opportunity to capture the decades of discovery of RNA biology to deliver small molecule chemical probes and lead medicines targeting RNA. Although RNA has been thought to not be broadly targetable with organic ligands, these advances suggest that this needs reassessment.

PHYSIOLOGY WITH CAGED COMPOUNDS

Prof. Graham Ellis-Davies - Icahn School of Medicine at Mount Sinai

Caged compounds are biologically inert, photolabile derivatives of biomolecules such as ATP, glutamate, IP3, etc. Covalent attachment of a photochemical protecting group to a crucial functionality generates the caged biomolecule, irradiation cuts the bond, allowing photostimulation of a receptor. This technique has been used for physiological studies of all cell types. I will present recent work on the development of new caged compounds that enable: (1) wavelength-selective uncaging of two biomolecules; (2) use of caged glutamate in vivo without off-target effects; (3) photostimulation of nicotinic acetylcholine receptors.

SMALL MOLECULES FROM THE HUMAN MICROBIOTA

Dr. Michael Fischbach - Stanford University

The human microbiome is linked to a range of phenotypes in the host, but it remains difficult to test causality and explore the mechanisms of these interactions. The ability to do perform single-strain and single-gene deletion experiments in the microbiome would be transformative for our ability to study molecular mechanisms. This seminar will describe our efforts to develop technology in two areas to make this possible: 1) Genetics: It is difficult to probe mechanism without genetics, and genetic tools exist for only ~10% of the bacterial species in the gut and skin microbiome. I will describe our efforts to develop systems for gene and operon insertion and deletion in common

bacterial taxa in the microbiome that are currently refractory to genetic manipulation. 2) Highly complex communities: There is a pressing need for model systems for the microbiome that are defined, but of an order of complexity that approaches the native state. I will discuss our work to build highly complex defined communities (100-200 bacterial species), make them stable upon transplantation into mice, and probe their function in vitro and in vivo.

OPTICAL TOOLS FOR MANIPULATING CLASSICAL AND ORPHAN CANNABINOID RECEPTORS

Prof. James Frank - Vollum Institute, Oregon Health & Science University

Cannabinoid receptors (CBRs) are G protein-coupled receptors (GPCRs) that are ubiquitously expressed in humans throughout the nervous system and periphery. The two main isoforms, CB1 and CB2, are inhibitory receptors which act via adenylyl cyclase and K^+ channels to decrease cellular excitability. More recently, however, stimulatory "orphan" GPCRs like GPR55 have also been shown to respond to endocannabinoid ligands. These increase intracellular Ca^{2+} levels via phospholipase C, and are expressed in many of the same cells/tissues as the classical CBR isoforms. This heterogeneity is especially critical in excitable cell populations such as neurons or pancreatic β-cells, where the subcellular localization, pharmacology and downstream signaling pathways of each CBR isoform remain poorly understood. To address this complexity, we have developed a number of small-molecule optical tools to place specific cannabinoid receptor isoforms under optical control. These include both photoswitchable and photocaged ligands, whose efficacy can be controlled on demand via an optical switch. We use these photo-controllable cannabinoids to probe the downstream mechanisms by which CBRs signal in excitable cell populations, with the goal of distinguishing the effects of classical vs. orphan receptor activity in modulating the secretion of hormones and neurotransmitters.

ARCHITECTURES AND MECHANISMS OF SIGNALING AT THE CHEMICAL SYNAPSES OF THE BRAIN

Dr. Eric Gouaux – Vollum Institute, Oregon Health & Science University

The Gouaux lab seeks to understand the molecular basis for signal transduction at chemical synapses in the central nervous system and at sites of mechanical-electrical transduction (MET) in hair cells of the inner ear. We strive to not only elucidate molecular structures of the neurotransmitter receptors and transporters at synapses, but we also aim to elaborate the composition and molecular organization of the MET complex. We apply a host of structural and biophysical approaches, including single particle and tomographic cryo-electron microscopy, together with fluorescence microscopy methods, biochemical methods, patch clamp electrophysiology and molecular biological manipulations.

A CHEMICAL BIOLOGY TOOLBOX FOR RNA POST-TRANSCRIPTIONAL MODIFICATION AND CAPTURE

Dr. Jennifer Heemstra - Emory University

Biomolecules are exquisitely adept at molecular recognition and self-assembly, enabling them to direct all of the processes that make life possible. These capabilities have been fine-tuned by billions of years of evolution, and more recently, have been harnessed in the laboratory to enable the use of biomolecules for applications beyond their canonical biological roles. The common thread that is

woven throughout our research program is the utilization of nucleic acid molecular recognition and self-assembly to generate functional architectures for biosensing and bioimaging. In the process of generating these functional nucleic acid systems, we place a high value on using our experimental results (both successes and failures) to gain a deeper understanding of the forces that drive interactions between nucleic acids and small molecules or proteins. We have developed RNA sequences that recognize specific small molecule fluorophores and catalyze covalent self-labeling, and research is ongoing to utilize these self-labeling ribozymes for imaging of RNA in living cells and transcript-specific capture of RNA-binding proteins. We have also demonstrated the modulation of EndoV activity to enable selective enrichment of inosine-containing RNAs, which we anticipate will lead to the identification of new A-to-I editing sites.

CHEMICAL BIOLOGY UNDER THE SKIN

Dr. Paul Heppenstall - Scuola Internazionale Superiore di Studi Avanzati, Italy

Pain and itch are worldwide health problems that exact substantial financial and societal costs. While enormous advances have been made in our understanding of the biological basis of pain, for the most part this has not been translated into new therapies for its treatment. A contributing factor to the lack of translation impact of pain research is that small molecules that target pain pathways are often unsuccessful because of redundancy in the system. Here I will discuss an alternative approach to treating pain and itch based upon achieving control of sensory neuron activity at the point where sensation is initiated in the skin. Key to the development of this approach has been a number of mouse genetic studies which have used opto and chemogenetics to explore the contribution of distinct subpopulations of sensory neuron to different types of pain and itch. Taking this further I will show how these genetic strategies can also be translated into pharmacological technologies by means of the ligands that bind to membrane receptors expressed on these distinct populations of neuron. I will describe how cargoes such as photosensitizers can be attached to these ligands and delivered in vivo in mice. Application of light to the skin then results in retraction of neurons from their end organs and long-term reversal of symptoms in animal models of chronic pain or itch. Finally, I will discuss how this technology can be applied to other cargoes with the ultimate aim of gaining optical and chemical control over neuronal activity, thus inhibiting pain at its source. Further I will show how these genetic strategies can also be translated into pharmacological technologies that allow us to gain optical and chemical control over neuronal activity using exogenous molecules. Finally, I discuss how this type of technology may have clinical potential for the development of new therapeutic strategies for treating itch and pain.

Questions:

1. What theories have been postulated to explain how sensation is encoded by the peripheral nervous system?
2. What are the mechanisms that could explain how an innocuous touch becomes painful after injury?
3. Why is it so difficult to translate basic research in pain and itch into new medicines?

Reviews:
https://www.cell.com/neuron/fulltext/S0896-6273(13)00710-1
Focus on Pain and itch (several reviews): https://www.nature.com/articles/nn.3644

NEUROPLASTICITY AND THE SWEETER SIDE OF CELL SIGNALING

Dr. Linda Hsieh-Wilson - Division of Chemistry and Chemical Engineering, California Institute of Technology

The field of chemical neurobiology is rapidly evolving and providing insights into the molecules and interactions involved in neuronal development, sensory perception, and memory storage. We will describe the development of new synthetic methods and chemical tools to understand how glycosaminoglycans contribute to neuroplasticity – the ability of the brain to adapt and form new neural connections. By combining synthetic organic chemistry, computational chemistry, cell biology, and in vivo biology, we have shown that specific sulfation motifs within these polysaccharides regulate signaling events that underlie processes such as axon regeneration, synaptic plasticity, and the formation of neural circuits.

ELUCIDATING HYPOTHALAMIC CIRCUITS CONTROLLING HOMEOSTATIC FUNCTIONS USING OPTOGENETICS

Prof. Martin J. Kelly – Oregon Health & Science University

Puberty and fertility are necessary for survival of the species, and these functions are highly dependent on hypothalamic peptidergic neuronal circuits. A recently discovered peptide, kisspeptin, has been shown to be a potent activator of gonadotropin-releasing hormone (GnRH) neurons, which form the final common pathway to regulate the pituitary-gonadal axis and ultimately fertility. It is well known that the episodic (pulsatile) release of GnRH is absolutely vital for reproductive success. With the discovery of kisspeptin, it has been hypothesized that the hypothalamic arcuate nucleus kisspeptin ($Kiss1^{ARH}$) neurons are the "pulse generator" and the neuropeptides co-expressed by these neurons are responsible for the pulsatile release pattern. However, it has been difficult to determine the cellular mechanism of pulse generation and how this is transmitted to GnRH neurons.

We characterized the cellular basis for synchronized $Kiss1^{ARH}$ neuronal firing using optogenetics, whole-cell electrophysiology, molecular pharmacology and single cell RT-PCR. $Kiss1^{ARH}$ neurons co-express neurokinin B (NKB), dynorphin and glutamate. High frequency (20 Hz) photostimulation of $Kiss1^{ARH}$ neurons evokes local co-release of the neuropeptides NKB and dynorphin, which synchronize neuronal firing via the Gq-coupled tachykinin 3 receptor (Tacr3) and Gi,o-coupled κ-opioid receptor, respectively. The light-evoked synchronous activity is also transmitted to rostral GnRH neurons via a trans-synaptic mechanism that involves glutamatergic input to preoptic Kiss1 neurons, which in turn excite GnRH neurons via kisspeptin release. Hence, $Kiss1^{ARH}$ neurons play a dual role of driving episodic secretion of GnRH through differential release of peptide and amino acid neurotransmitters to coordinate reproductive function. As proof of principle, we expressed the genetically-encoded calcium indicator GCamp6 in $Kiss1^{ARH}$ neurons and found that the Tacr3 agonist senktide caused synchronous calcium oscillations in the $Kiss1^{ARH}$ neurons. Moreover, these "command" neurons of reproductive function are highly regulated by gonadal (17β-estradiol) and metabolic (insulin) hormones that allow them to coordinate reproductive function with energy

homeostasis. Therefore, our cellular characterization of $Kiss1^{ARH}$ neuronal excitability has allowed a deeper understanding of the critical role of peptidergic neurotransmission in physiological and pathophysiological states.

GLYCANS AS MICROBIAL IDS

Dr. Laura Kiessling - Massachusetts Institute of Technology

As animals living amongst microbes, we need to distinguish between microorganisms that are benign or beneficial versus those that are pathogenic. Our health depends on a maintaining a functional microbiome while avoiding the propagation of pathogenic microbes. As microbial hosts, we therefore must have mechanisms to influence which microbes stay and which must go. To this end, our group is focusing on a prominent feature of the cell's exterior—the carbohydrate coat. From humans to fungi to bacteria, virtually all cells on Earth possess a carbohydrate coat. One important role of this coat is to serve as an identification card. Our group has been examining the role of carbohydrate-binding proteins, lectins, in influencing our microbiota and in immune defense. This seminar will focus on understanding the basis of carbohydrate-protein interactions and how they are used to influence microbes. We envision that our findings can lead to alternative means to combat pathogens, methods for rapid approaches to ID microbiota, and the development of new strategies to regulate microbiome composition to promote human health.

NEW BIOLOGICAL FINDINGS WHICH WERE REVEALED BY DESIGNED FLUORESCENT PROBES

Prof. Kazuya Kikuchi - Osaka University

One of the great challenges in the post-genome era is to clarify the biological significance of intracellular molecules directly in living cells. If we can visualize a molecule in action, it is possible to acquire biological information, which is unavailable if we deal with cell homogenates. One possible approach is to design and synthesize chemical probes that can convert biological information to chemical output. In this talk, molecular design strategies for fluorescence imaging probes are introduced.

Intravital imaging by two-photon excitation microscopy (TPEM) has been widely utilized to visualize cell functions. The combination of the rationally designed small molecular probes with a fluorescent protein as a reporter of cell localization enabled quantitation of osteoclast activity and time-lapse imaging of its in vivo function.

Intracellular translocation of proteins is an important mechanism for regulating physiological function of proteins. Fluorescence imaging is a powerful technique to obtain real-time information on protein translocation. We have recently developed an imaging technique using PYP-tag, which is a small protein tag (14 kDa) derived from purple bacteria. The key advantage of this technique is the enhancement of fluorescence intensity upon labeling reactions. GLUT4 is an N-glycosylated transmembrane protein, which plays a central role in maintaining glucose homeostasis. By taking advantage of our protein labeling system, we revealed the role of the N-glycan in the intracellular trafficking.

INTESTINAL FUCOSE, IN SICKNESS AND IN HEALTH

Dr. Jennifer Kohler - University of Texas Southwestern

The surface of all human cells is covered with a dense coating of glycosylated molecules that dictates the interactions of the cells with their environment. Nowhere is this more true than within the gastrointestinal tract, where the epithelial surface is coated with a mucus layer that is a critical mediator of communication with the diverse community of microorganisms that form the gut microbiome. The intestinal epithelial mucus layer is composed primarily of heavily O-glycosylated glycoproteins, called mucins, along with additional N-linked glycoproteins and glycolipids. A defining characteristic of the mucus layer is the presence of the fucose monosaccharide. Fucosylation of the intestinal epithelial occurs in a interleukin 22 (IL-22)-dependent manner in response to colonization with certain species of commensal bacteria, and fucose serves as nutrient for many gut microbes. However, pathogens also take advantage of intestinal fucosylation, and use recognition of fucosylated epitopes as a means to attack host cells. In my seminar, I will discuss roles for intestinal fucose in both normal physiology and in infectious disease. First, we examined the host cell receptors for cholera toxin, the secreted protein toxin that causes the symptoms of cholera. While cholera toxin has long been known to bind to the glycolipid GM1 with high affinity, we found little GM1 present in human intestinal epithelial cells. Rather, an important factor in binding of cholera toxin to human cells appears to be recognition of fucosylated structures, which bind to the toxin in a secondary glycan binding site. We also found that fucosylated molecules present in high abundance in human milk can competitively inhibit cholera toxin binding to intestinal epithelial cells. Current efforts focus on identifying the fucosylated glycoconjugates present in human intestinal epithelia and delineating the relative contributions of GM1 and fucosylated molecules to host cell intoxication. In a second project, we examined the effects of the cytokine IL-22 on glycosylation of human intestinal epithelia cells grown in culture. We observed an increase in fucosylation, with the majority of the fucose found on mucin-type glycoproteins. This increase in fucosylation was not accompanied by changes in the transcript level of any fucose-related genes. Rather, our data suggest that changes in the expression of other glycosyltransferases may alter the mucin glycoprotein structure to allow for increased fucosylation. Current efforts focus on defining the mechanism underlying the IL-22-dependent change in fucosylation, and investigating the impact on susceptibility to gut pathogens and on the ability of the mucus layer to promote a healthy microbiome.

SPECIFIC MODULATORS OF PROTEIN PHOSPHATASE-1 AND THEIR APPLICATION IN HEART FAILURE

Dr. Maja Köhn - University of Freiburg, Signaling Research Centers BIOSS and CIBSS and Faculty of Biology

Protein serine/threonine-specific phosphatases have in the past been considered to be housekeeping enzymes, undruggable, and challenging to study due to their multiple roles and the conservation of the catalytic subunits. However, this view is currently changing. Of these, protein phosphatase-1 (PP1) is an important ubiquitous phosphatase that is estimated to remove phosphate groups from about a third of all phosphorylated serines and threonines in eukaryotic cells, counteracting more than a hundred kinases. PP1 catalytic subunit (PP1c) has broad substrate

specificity but is restrained in vivo by numerous PP1c-interacting proteins that impart high substrate specificity to it and function for example as activity-modulating or localization-determining factors. These so-called PP1-holoenzymes play roles in many different diseases such as cancer, diabetes, and cardiovascular diseases. The lack of selective modulators of PP1 has in the past been a limiting factor in its research. We have addressed this challenge by designing peptides that target PP1c and disrupt its protein–protein interactions with regulatory proteins, leading to the release of free, active PP1c inside cells. I will report on the probe development and describe their application to study and target PP1 activity in the pathomechanism of heart failure.

FROM SINGLE-MOLECULE IMAGING TO THE BRAIN: A CIRCUITOUS ROUTE TO NEW NEURAL ACTIVITY INDICATORS

Dr. Luke Lavis - Janelia Research Campus, Howard Hughes Medical Institute

Small molecules remain important tools to probe or perturb biological systems. Designing chemical reagents for modern neuroscience remains a significant challenge, however, since the brain is the most complex and least accessible organ in the body. My lab initially focused on molecular tools for neuroscience, but these efforts largely failed. Frustrated by the brain, we instead began developing reagents for cell biology with the goal of creating bright, cell-permeable dyes for single-molecule imaging. Inspired by computational experiments, we discovered that replacing the *N*,*N*-dimethylamino substituents in the classic dye tetramethylrhodamine with four-membered azetidine rings greatly improved brightness and photostability. The novel substitution is generalizable to fluorophores from different structural classes and enables fine-tuning of the dyes' spectral and chemical properties. This effort yielded a palette of fluorophores useful in live-cell imaging experiments and we have since turned our focus back to the brain, learning that these dyes can also be delivered to neurons *in* vivo. This allows the construction of hybrid small-molecule:protein sensors with substantially higher brightness and photon yields, facilitating new functional imaging experiments to measure changes in voltage or [Ca^2+].

FRIENDS OR FOES? NEWS ON OLD NUCLEOTIDES

Prof. Andreas Marx - University of Konstanz

Cells contain several minor nucleotides in addition to the canonical mono-, di- and triphosphates. Among these, various dinucleoside polyphosphates (Np_nN', n = 2–7) are found. The dinucleoside polyphosphates consist of two nucleoside moieties, which are linked by a polyphosphate chain at the respective 5'-hydroxyl groups. These molecules are present in prokaryotic as well as eukaryotic cells. Despite being known for 50 years the functions of dinucleoside polyphosphates are still unclear. The most abundant nucleotides of such kind are diadenosine tri- and tetraphosphate (Ap_3A and Ap_4A, respectively). Interestingly, their cellular concentrations vary dependent on cell type and environmental factors such as stress and increase from a nanomolar to a micromolar range. Therefore, it was postulated, that that these nucleotides serve as a cellular signal ('alarmones') of stressors and thus, are involved in stress adaptation processes of cells. However, their exact role is yet to be specified: Are they intracellular signalling molecules that trigger adaptation in response to cellular stress or are they metabolic side products that potentially harm the cell?

In the presentation, new insights into the role and function of Ap_3A and Ap_4A will be presented and discussed.

CHEMICAL PROTEOMIC PLATFORMS TO EXPOSE AND EXPLOIT NOVEL METABOLIC SIGNALS IN DISEASE

Prof. Raymond Moellering - Institute for Genomics and Systems Biology, University

Biological systems are inherently and profoundly heterogeneous, both at the molecular level (e.g. encoded proteins existing in distinct posttranslational modification states and macromolecular complexes) and the cellular level (e.g. intra- and intercellular localization of biomolecules). Despite growing awareness and appreciation for this level of molecular complexity in living systems, most studies still rely on reductionist strategies to interrogate the proteome, owing to the significant technical challenges associated with studying protein structure, function and organization in native environments. We believe that in order to understand molecular information flow under basal or diseased conditions we must be able to probe biomolecular function and organization in native environments across scales of space and time. Therefore, innovation in the development probes and technology platforms is needed. In the first part of this talk, I will describe the development of new chemical probes and complementary proteomic platforms that enable quantitative detection of protein functional states - including enzymatic activity, protein-protein interaction partners, and the functional consequences of posttranslational modification states - in live cells. In the second half of this talk, I will describe how integration of these platforms can be harnessed to discover new roles for endogenous metabolites as intracellular signals in normal and diseased contexts, as well as the potential to regulate these signals for therapeutic benefit. Both halves of the talk will emphasize the integration of chemical proteomic platforms as a discovery engine to identify novel targets for diagnostic and therapeutic development in human disease.

PAINTING CHROMATIN WITH SYNTHETIC PROTEIN CHEMISTRY

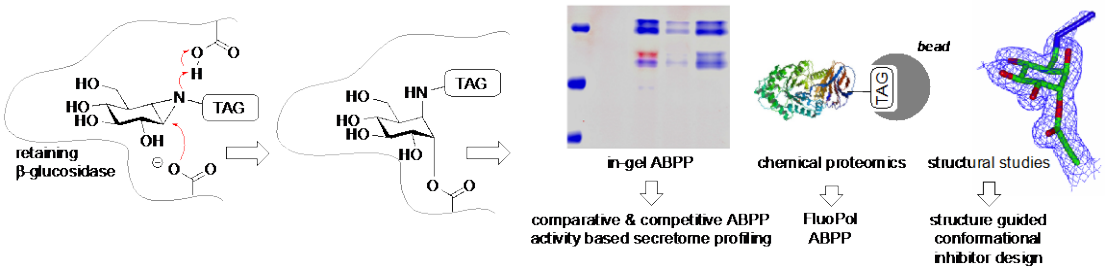
Dr. Tom Muir - Princeton University

Understanding protein function is at the heart of experimental biology. Perhaps one of grandest contemporary challenges in this area is to catalogue and then functionally characterize protein post-translational modifications (PTMs). Modern analytical techniques reveal that most, if not all, proteins are modified at some point; it is nature's way of imposing functional diversity on a polypeptide chain. Understanding the structural and functional consequences of all these PTMs is a devilishly hard problem. While standard molecular biology methods are of limited utility in this regard, modern protein chemistry has provided powerful methods that allow the detailed interrogation of protein PTMs. In this lecture, I will highlight the use of high-throughput methods for studying the role of PTMs in regulating aspects of chromatin biology. In particular, I will discuss how histone modifications, and cancer-associated mutations, impact the activity of chromatin modifying enzymes and chromatin remodeling machines.

ACTIVITY-BASED GLYCOSIDASE PROFILING IN BIOMEDICINE AND BIOTECHNOLOGY

Dr. Hermen Overkleeft - Leiden Institute of Chemistry, Leiden University

Activity-based protein profiling (ABPP) is a rapidly emerging field in chemical biology research. Enzymes that employ a mechanism in processing their substrate that involves formation of a covalent enzyme-intermediate adduct can be blocked by mechanism-based suicide inhibitors: compounds that react within the enzyme active site to form a covalent and irreversible adduct. Introduction of a reporter moiety ('TAG' in the below picture) yields an activity-based probe (ABP) through which enzyme activities can be discovered (comparative ABPP) and the efficacy enzyme inhibitors in complex biological systems analyzed (competitive ABPP).



Our work on ABPP development focuses on retaining glycosidases: hydrolytic enzymes able to cleave interglycosidic linkages and that do so through the formation of covalent enzyme-substrate intermediates. Configurational and functional analogues of the natural product and mechanism-based retaining beta-glucosidase inhibitor, cyclophellitol, prove to be highly versatile tools to study retaining glycosidases of various nature and origin in relation to human health and disease, but also in the field of biotechnology. In this lecture the current state in the design, synthesis and application of synthetic cyclophellitol derivatives in studying retaining glycosidases will be presented. Discussed subjects will include 1) diagnosis of human lysosomal exoglycosidases in relation to lysosomal storage disorders; 2) glycosylation of cyclophellitol derivatives top arrive at retaining endoglycosidase ABPs and 3) application of glycosidase ABPs in the functional profiling of fungal secretomes for the discovery of glycosidases for biotechnology application.

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RIBOGLOW: A NEW PLATFORM FOR TRACKING RNA MOLECULES IN LIVE MAMMALIAN CELLS

Dr. Amy Palmer - University of Colorado, Bolder

The complex spatiotemporal dynamics of messenger RNAs and non-coding RNAs affect virtually all aspects of cellular function. In addition to serving as the central intermediary between DNA and proteins, RNAs regulate gene expression at multiple levels, play roles in epigenetic regulation and genome organization, and serve as physical scaffolds to assemble and integrate macromolecular complexes. Despite the importance of RNA in biology and growing evidence of complex and dynamic localization patterns, robust tools for visualizing RNA molecules in live cells are limited. Recently, we developed an RNA imaging platform using the cobalamin riboswitch as an RNA tag and a series of molecular probes containing cobalamin as a fluorescence quencher. This highly modular 'Riboglow' platform leverages different color fluorescent dyes, linkers and riboswitch RNA tags to elicit fluorescent turn-on upon binding RNA. We have demonstrated the ability of Riboglow probes to track recruitment of mRNA and small non-coding RNA to ribonucleoprotein (RNP) granules in live mammalian cells, including both stress granules and U-bodies. In addition, we have demonstrated that with 12-copies we can detect and track single mRNA molecules undergoing diffusion, splicing and engaged in translation. In this talk I will introduce the Riboglow platform and discuss our current approaches for creating orthogonal tags for simultaneous imaging of multiple RNA species, efforts to further engineer fluorescent turn on, and strategies for assessing whether RNA tags perturb underlying properties of the RNA.

O-GLCNAC AS A MULTIFACETED INHIBITOR OF AMYLOID AGGREGATION

Dr. Matthew Pratt - University of Southern California

The glycosylation of serine and threonine residues by the monosaccharide N-acetylglucosamine, termed O-GlcNAc modification, is an important and abundant posttranslational modification in plants and animals. This intracellular modification is dynamic and responds to a variety of biological inputs, including metabolism and cellular stress. One of the key functions of O-GlcNAcylation appears to be the inhibition of protein aggregation and protection of cells from the associated toxicity. However, the mechanisms by which O-GlcNAc contributes to this protective phenotype are still somewhat mysterious, due to a lack of biological tools to test the effects of specific O-GlcNAc modifications. To overcome this fundamental limitation, my lab uses protein semi-synthesis to prepare homogeneously O-GlcNAcylated proteins for biochemical study. Here, I will present our application of this technology to demonstrate that O-GlcNAc inhibits protein aggregation through at least two mechanisms: the physical blockage of aggregate formation and the activation of heat shock proteins. These results further support the exploitation of O-GlcNAc to slow the progression of neurodegenerative diseases.

CHEMICAL TOOLS TO STUDY THE DYNAMICS OF THE TRANSCRIPTOME

Dr. Matthew Simon - Yale University

The expression of different cellular RNAs is regulated by controlling the rates of synthesis and decay. This regulation occurs at many steps in RNA metabolism, starting with the accessibility of transcription start sites in chromatin and extending to polymerase dynamics, RNA processing, transport and degradation. Teasing apart the different strategies a cell uses to regulate gene expression requires methods to monitor the flux of RNAs through these many regulated steps. Cellular RNAs can be monitored through metabolic labeling using thiolated nucleosides including 4-thiouridine (s^4U) and 6-thioguanosine (s^6G). We have developed chemistry in two areas to provide new insight into RNA metabolism. First, we have developed improved methods to biochemically enrich these metabolically labeled transcripts, reducing bias and increasing yields. Second, we have developed chemistry to recode the hydrogen bonding pattern of sU and s^6G nucleotides in metabolically labeled RNA, converting them into cytosine and adenosine analogues, respectively. This TimeLapse-seq approach allows the identification of newly transcribed RNAs directly in a sequencing experiment without the need for biochemical enrichment. Together these approaches can be used to study RNA metabolism across a wide range of time scales providing new insight into regulated gene expression.

LIVE CELL IMAGING AND CELLULAR REPROGRAMMING IN IMMUNO-ENGINEERING

Dr. Peter Yingxiao Wang - University of California, San Diego

Genetically-encoded biosensors based on fluorescence proteins (FPs) and fluorescence resonance energy transfer (FRET) have enabled the specific targeting and visualization of signaling events in live cells with high spatiotemporal resolutions. Single-molecule FRET biosensors have been successfully developed to monitor the activity of a variety of signaling molecules, including tyrosine/serine/threonine kinases. We have a developed a general high-throughput screening (HTS) method based on directed evolution to develop sensitive and specific FRET biosensors. We have first applied a yeast library and screened for a mutated binding domain for phosphorylated peptide sequence. When this mutated binding domain and the peptide sequence are connected by a linker and then concatenated in between a pair of FRET FPs, a drastic increase in sensitivity can be achieved. HTS integrated with directed evolution and next generation sequencing (NGS) was also employed in mammalian cells to develop sensitive and specific FRET biosensors. It has been increasingly clear that controlling protein functions using lights and chemical compounds to trigger allosteric conformational changes can be applied to manipulate protein functions and control cellular behaviors4-8. We have also integrated with lights and ultrasound to manipulate the molecular activation of genes and enzymes, which allowed us to control the cellular functions of immunocells with high precision in space and time.

#101 - THE LC8-RAVP ENSEMBLE STRUCTURE EVINCES A ROLE FOR LC8 IN REGULATING LYSSAVIRUS POLYMERASE FUNCTIONALITY

Prof. Elisar Barbar - Oregon State University

Dr. Nathan Jespersen – Oregon State University

The replication and transmission of a virus is dependent on a multitude of interactions between host and viral proteins. In general, proteins that are frequently co-opted by viruses are key to viral growth or host defense. One such protein is the highly conserved hub LC8, which binds to multiple viral partners. The rabies virus phosphoprotein, the Ebola virus VP35, and the human immunodeficiency virus integrase, represent a small percentage of the more than 100 proteins known or predicted to bind LC8 in a wide array of cellular systems. LC8 binds its partners at a well-characterized and conserved recognition motif. Intriguingly, a null mutation in the LC8 binding motif of the rabies virus phosphoprotein (RavP) results in completely non-lethal viral infections in mice, compared to 100% lethality for rabies infections involving wild type RavP. Cell-based experiments suggest roles for LC8 in primary transcription and intracellular localization (43); however molecular level descriptions of the role of LC8 in viral lethality have been hampered by the structural complexity of the multi-domain, partially disordered RavP.

In this work, we examine the molecular role LC8 plays in viral lethality. We show that RavP and LC8 co-localize in rabies infected cells, and that LC8 interactions are essential for efficient viral polymerase functionality. NMR, SAXS, and molecular modeling demonstrate that LC8 binding to a disordered linker adjacent to an endogenous dimerization domain results in restrictions in RavP domain orientations. The resulting ensemble structure of RavP-LC8 tetrameric complex is similar to that of a related virus phosphoprotein that does not bind LC8, suggesting that with RavP, LC8 binding acts as a switch to induce a more active conformation. The high conservation of the LC8 motif in *Lyssavirus* phosphoproteins and its presence in other analogous proteins such as the Ebola virus VP35 evinces a broader purpose for LC8 in regulating downstream phosphoprotein functions vital for viral replication.

#12 - CHRONIC EXPOSURE TO COMPLEX METAL OXIDE NANOPARTICLES ELICITS RAPID BACTERIAL RESISTANCE

Prof. Erin Carlson - University of Minnesota, Twin Cities

Engineered nanoparticles are incorporated into numerous emerging technologies because of their unique physical and chemical properties. Many of these properties facilitate novel interactions, including both intentional and accidental effects on biological systems. Silver-containing particles are widely used as antimicrobial agents and recent evidence indicates that bacteria rapidly become resistant to these nanoparticles. Much less studied is the chronic exposure of bacteria to particles that were not designed to interact with microorganisms. For example, previous work has demonstrated that the lithium intercalated battery cathode nanosheet, nickel manganese cobalt oxide (NMC), is cytotoxic and causes a significant delay in growth of *Shewanella oneidensis* MR-1 upon acute exposure. We found that *S. oneidensis* MR-1 rapidly adapts to chronic NMC exposure and is subsequently able to survive in much higher concentrations of

these particles, providing the first evidence of permanent bacterial resistance following exposure to nanoparticles that were not intended as antibacterial agents. We also found that when NMC-adapted bacteria were subjected to only the metal ions released from this material, their specific growth rates were higher than when exposed to the nanoparticle. As such, we provide here the first demonstration of bacterial resistance to complex metal oxide nanoparticles with an adaptation mechanism that cannot be fully explained by multi-metal adaptation. Importantly, this adaptation persists even after the organism has been grown in pristine media for multiple generations, indicating that *S. oneidensis* MR-1 has developed permanent resistance to NMC. Full characterization of these mutant organisms, as well as evaluation of the scope of organisms capable of evolving resistance are currently underway.rahul

#42 - A CHEMOPROTEOMIC PLATFORM FOR PROFILING THE CELLULAR INTERACTOME OF PROTEIN KINASES

Mr. Linglan Fang - University of Washington - Seattle

Ms. Sujata Chakraborty - University of Washington - Seattle

Ms. Emily Dieter - University of Washington

Mr. Zachary Potter - University of Washington - Seattle

Prof. Dustin Maly - University of Washington - Seattle

Small molecule inhibitors often only block a subset of the cellular functions of their protein kinase targets and, in many cases, lead to phenotypic effects that are not well understood. To systematically characterize how inhibited protein kinases influence cellular behavior, we developed a chemoproteomic method for interrogating the cellular localization and interactomes of kinases. By developing a set of selective inhibitors of Src kinase that contain a trans-cyclooctene (TCO) click handle, we leveraged this moiety's rapid and mild reaction with tetrazines to enrich and characterize the proteins bound to this multi-domain signaling enzyme. Using a streamlined quantitative proteomics platform with our TCO probe set, we observed that a kinase's cellular interactions can be reprogrammed by modulating its global conformation. Furthermore, we observed that the signaling state of the cell has a profound effect on the interactomes of kinases. We also demonstrated that the TCO-conjugated probes can be used as a part of a proximity ligation assay to study Src's cellular localization and interactions in *situ* with confocal microscopy.

COMPUTATIONALLY-DRIVEN, STRUCTURE-BASED DRUG DISCOVERY

Dr. Leah Frye - Schrödinger

With access to a growing number of high quality protein x-ray, NMR, and cryo-EM structures, as well as advanced physics-based computational models, it is possible to greatly accelerate all stages of the drug discovery process from target selection to clinical candidate. This presentation will discuss prospective, real world examples of the use of structurally-driven computational approaches to assess the druggability of potential ligand binding sites, identify novel lead compounds via scaffold hopping, and utilize free energy perturbation (FEP) and AI methods to predict relative ligand binding affinity allowing for the selection of optimal compounds for synthesis

that address potency and/or selectivity issues. This approach has allowed us to rapidly progress a number of programs to the development candidate stage in the past few years.

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

Dr. Rahul Singh Kathayat - The University of Chicago

Ms. Yang Cao - The University of Chicago

Mr. Tian Qiu - The University of Chicago

Ms. Saara-Anne Azizi - The University of Chicago

Prof. Bryan Dickinson - The University of Chicago

Keywords. *S*-palmitoylation, Post-translational modification (PTM), Acyl-protein thioesterases (APTs), Depalmitoylation probes (DPPs), Spatially-constrained probes, Mitochondria, APT1, ABHD10, Mitochondrial redox homeostasis, PRDX5

S-palmitoylation is an abundant and reversible lipid post-translational modification (PTM) that participates in regulating protein localization, trafficking and therefore have consequences in cellular signaling.[1,2] Recently, we developed fluorescence-based turn-on depalmitoylation probes (DPPs)[3,4] and mitochondrial-targeted DPPs (mitoDPPs)[5] to probe APTs activity in the cytosol and mitochondria of live cells, respectively. Using mitoDPPs, we established that there is active *S*-depalmitoylation in mitochondria, and also shown that it responds dynamically to external cues like palmitate stress and genetic-based alterations in local palmitoyl-CoA reserves. Furthermore, we established that APT1, which was previously known as an exclusively cytosolic protein, is also localized in mitochondria.

Our current research focuses on investigating the regulation and functional consequences of *S*-palmitoylation in mitochondria. Here, we show that mitochondrial antioxidant buffering capacity diminishes on perturbing the "erasers" of this PTM, acyl protein thioesterases (APTs), using either pan-active inhibitors or a new mitochondrial-targeted APT inhibitor. However, this effect was not mediated by APT1, the only known mitochondrial APT, but rather by another mitochondrial resident protein, ABHD10, for which no endogenous function is yet known.[6] We demonstrate that ABHD10 is a new member of the *S*-palmitoylation eraser family.[6] We then identify a key cellular antioxidant protein, peroxiredoxin 5 (PRDX5), as the first target of ABHD10 *S*-depalmitoylase activity.[6] Furthermore, we show 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 assigning more substrates and functions to mitochondrial *S*-depalmitoylation. Additionally, the new mitochondrial-targeted APT inhibitor will help *S*-palmitoylation research community to further explore function and regulation of *S*-depalmitoylation in mitochondria.

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#132 - FUNCTIONAL PROFILING OF PROTEASES THAT MODULATE HOST-BACTERIAL INTERACTIONS IN THE GUT

Dr. Markus Lakemeyer - Stanford University

Ms. Laura Keller - Stanford University

Mr. Will Van Treuren - Stanford University

Prof. Justin Sonnenburg - Stanford University

Prof. Matthew Bogyo - Stanford University

Proteolysis is central to numerous cellular processes in the human body including development, differentiation and immune response. In the human gastrointestinal (GI) tract, proteases such as trypsin and chymotrypsin are highly prevalent, where they mediate digestive functions, among others. Excessive proteolytic activity in the colonic lumen is associated, for example, with inflammatory bowel disease (IBD) or irritable bowel syndrome (IBS). While the exact mechanisms remain elusive, dysregulated signaling of protease-activated receptors (PARs) has been associated with these and additional conditions. PARs describe a family of four G-protein coupled receptors (GPCRs) that are activated upon proteolytic cleavage of extracellular N-terminal peptide sequences. PARs are highly prevalent in the lumen, where they influence barrier permeability, among others. The human microbiome produces a plethora of metabolite and protein products that are expected to be key drivers of health and disease. Despite the co-occurrence of PARs and commensal bacteria in the GI tract, the impact of bacterial proteases on gut homeostasis and pathogenesis remains poorly understood. In this project, I aim to profile proteases from commensal bacteria that modulate PAR signaling. A phenotypic screen using conditioned media from a library of 100 commensal strains revealed three strains that secrete PAR2-activating proteases. Next, we will use chemoproteomic profiling using molecular probes and mass spectrometry to identify the responsible enzymes. Recombinantly expressed proteases will be biochemically characterized and screened for selective small molecule inhibitors. Ultimately, recombinant enzymes, tailored inhibitors and genetically manipulated bacterial strains will be applied to reveal the biological importance of commensal proteases on PAR signaling, additional cellular functions and general gut homeostasis.

#98 - AN ENGINEERED ENZYME TO ALLEVIATE REDUCTIVE STRESS IN MITOCHONDRIAL DISEASE

Dr. Anupam Patgiri - Massachusetts General Hospital/Harvard Medical School/Broad Institute

Dr. Owen Skinner - Massachusetts General Hospital/Harvard Medical School/Broad Institute

Dr. Yusuke Miyazaki - Massachusetts General Hospital

Dr. Grigorij Schleifer - Massachusetts General Hospital

Dr. Eizo Marutani - Massachusetts General Hospital

Mr. Hardik Shah - Massachusetts General Hospital

Dr. Rohit Sharma - Massachusetts General Hospital/Harvard Medical School/Broad Institute

Dr. Russell Goodman - Massachusetts General Hospital/Harvard Medical School/Broad Institute

Dr. Tsz-Leung To - Massachusetts General Hospital/Harvard Medical School/Broad Institute

Dr. Xiaoyan Bao - Massachusetts General Hospital/Harvard Medical School/Broad Institute

Dr. Fumito Ichinose - Massachusetts General Hospital

Dr. Warren Zapol - Massachusetts General Hospital

Dr. Vamsi Mootha - Massachusetts General Hospital

The mitochondrion produces the majority of cellular ATP in a process called oxidative phosphorylation (OXPHOS) that uses the mitochondrial electron transport chain (ETC). A functional ETC is also necessary for numerous other important cellular processes like amino acid and nucleotide biosynthesis, fatty acid oxidation, and cell proliferation and death. Diseases associated with ETC dysfunction affect multiple organ systems and there is currently no treatment for these disorders. Although the lack of OXPHOS-derived ATP is classically considered as the main culprit in ETC deficiency, recent studies suggest that an elevated intracellular $NADH/NAD^+$ ratio (known as "reductive stress") caused by ETC dysfunction could also significantly contribute to the disease pathology. At a biochemical level, reductive stress inhibits multiple essential NAD^+-coupled pathways, including glycolysis which is the major source of ATP in cells with dysfunctional mitochondria. Therefore, we hypothesize that alleviation of reductive stress in mitochondrial disease could be therapeutic. Unfortunately, we currently do not have strategies to correct tissue $NADH/NAD^+$ ratios in animal models to test this hypothesis. To address this unmet need, we have developed an extracellular enzyme (named "LOXCAT") with the aim to lower the blood lactate/pyruvate ratio by converting lactate into pyruvate to then secondarily normalize disease-associated elevations in the tissue $NADH/NAD^+$ ratio. We designed LOXCAT based on the observation that the extracellular lactate/pyruvate ratio is in equilibrium with the intracellular $NADH/NAD^+$ ratio via the lactate dehydrogenase reaction in the cytosol and that ETC dysfunctions increase the blood lactate/pyruvate ratio in response to an increased tissue $NADH/NAD^+$ ratio. LOXCAT is an engineered fusion of enzymes lactate oxidase (LOX) and catalase (CAT), and it produces pyruvate and water from lactate and oxygen. Our proof of concept cell culture experiments showed that LOXCAT added to the media of cells with mitochondrial dysfunction significantly decreased the extracellular lactate/pyruvate ratio and normalized the intracellular $NADH/NAD^+$ ratio. Such mitigation in reductive stress allowed these cells to restore glycolytic ATP production to meet the cellular energy demand and rescue proliferative defects. To test *in vivo* efficacy, we injected the LOXCAT into the tail vein of a drug-induced mouse model of mitochondrial disease. We found that LOXCAT blunted the drug-induced increase in the blood lactate/pyruvate ratio and thereby normalized the brain and heart $NADH/NAD^+$ ratios (Patgiri *et al*, *Nat Biotech* 2019, accepted in principle). Significantly, we showed for the first time that the circulating lactate/pyruvate ratio functions as a systemic regulator of the tissue $NADH/NAD^+$ ratio. We are currently testing LOXCAT in genetic mouse models of mitochondrial disease. Together, LOXCAT lays the foundation for a novel class of therapeutics that targets circulating metabolites to impact tissue redox homeostasis in mitochondrial disease.

Reference:

Patgiri, A.; Skinner, O. S.; Miyazaki, Y.; Schleifer, G.; Marutani, E; Shah, H.; Sharma, R; Goodman, R. P.; To, T-L.; Bao, X. R.; Ichinose, F; Zapol, W. M.; and Mootha, V. K. (2019) "An engineered enzyme that directly targets circulating lactate to alleviate intracellular reductive stress" **Nature Biotechnology** ("*accepted in principle" for publication*)

#74 - SPLIT FLUORESCENT REPORTERS WITH RAPID AND REVERSIBLE COMPLEMENTATION

Dr. Alison Tebo - Sorbonne Université

Prof. Arnaud Gautier - Sorbonne Université

Interactions between proteins play an essential role in metabolic and signaling pathways, cellular processes and organismal systems. We developed splitFAST, a fluorescence complementation system for the visualization of transient protein-protein interactions in living cells. Engineered from the fluorogenic reporter FAST (Fluorescence-Activating and absorption-Shifting Tag), which specifically and reversibly binds fluorogenic hydroxybenzylidene rhodanine (HBR) analogs, splitFAST displays rapid and reversible complementation, allowing the real-time visualization of both the formation and the dissociation of a protein assembly. Here, we report the development of novel FAST-based split reporters (i) with emission properties in the far-red, and (ii) with orthogonal spectral and complementation properties for the detection of multiple interactions at the same time.

Reference:
Tebo, A. G. and Gautier, A. *Nat. Commun.* (2019) 2822.

#38 - VISUALIZING ENDOGENOUS OPIOID RECEPTORS IN LIVING NEURONS USING LIGAND-DIRECTED CHEMISTRY

Dr. Seksiri Arttamangkul - Oregon Health & Science University

Dr. Andrew Placzek - Oregon Health & Science University

Ms. Emily Platte - Oregon Health & Science University

Dr. Haihong Jin - Oregon Health & Science University

Dr. William Birdsong - Oregon Health & Science University

Prof. Thomas Murray - Creighton University

Dr. Kenner Rice - NIDA

Prof. David Farrens - Oregon Health & Science University

Prof. John Williams - Oregon Health & Science University

Identifying single neurons that have functional opioid receptors for cellular, synaptic and system actions of opioids is a key to advance the understanding of the opioid system. This study used a chemical approach known as “traceless affinity labeling” to develop a probe to fluorescently tag opioid receptors. Naltrexamine, an opioid antagonist, was chosen for the pharmacphore to link to a cleavable reactive acylimidazole and fluorescent dye. Fluorescent naltrexamine acylimidazole (NAI-A594) was synthesized. Once NAI-A594 bound to the receptors, the acylimidazole could react with nucleophiles of amino acid side chains on the receptors allowing the attachment of the fluorescent dye to the receptor and concurrently freed naltrexamine. The reaction was selective and labeled opioid receptors in multiple areas from mouse and rat brains. This method offers an advantage for visualization of living opioid-sensitive neurons in heterogeneous populations of the brain without loss of function. The ability to locate endogenously expressed receptors in living tissues will aide considerably in establishing the distribution and physiological role of opioid receptors in the CNS of wild type animals.

#115 - CLINICAL TRANSLATION OF NERVE-SPECIFIC FLUOROPHORES FOR IMAGE-GUIDED NERVE SPARING SURGICAL PROCEDURES

Dr. Connor Barth - Oregon Health & Science University

Dr. Summer Gibbs - Oregon Health & Science University

Dr. Lei Wang - Oregon Health & Science University

Dr. Adam Alani - Oregon State University

Dr. Vidhi Shah - Oregon State University

Dr. Alexander Antaris - Intuitive Surgical Inc.

Dr. Jonathan Sorger - Intuitive Surgical Inc.

Iatrogenic nerve injury is one of the most feared complications of surgery, with up to 600,000 patients affected annually. At present there is no technology to improve visual recognition of nerve tissue during surgery, and surgeons largely rely on anatomical knowledge to locate small or buried nerves invisible to the naked eye. We have developed first-in-kind targeted near-infrared (NIR) fluorophores that label nerve tissue with high affinity for direct nerve visualization during fluorescence-guided surgery. To date the most promising fluorophores are IT01-08 and IT05-75, which demonstrate high nerve signal to all background tissues (e.g., muscle, adipose, vasculature, fascia, etc.) following intravenous administration in rodents and swine. Notably, our selected fluorophores have widely varied pharmacokinetics (PK)

profiles, which could serve a range of surgical applications. This is an important point as iatrogenic nerve damage can occur in all surgical specialties including general, thoracic, cardiac, urologic, plastic, colorectal, spinal, neuro-, orthopedic and otolaryngologic surgery. Use of the fast-acting IT01-08 could benefit short procedures such as inguinal hernia repair (45 min), while IT05-75 may be applied to longer surgeries such as bilateral neck dissections (6-8 hrs). We have developed clinically viable formulation strategies for intravenous delivery of these agents and characterized their safety and pharmacological profiles. FDA-approved micelle and cyclodextrin formulations efficiently delivered these agents with excellent safety profiles. Pharmacokinetic, dose response, and biodistribution studies have quantified each agent's *in vivo* performance and identified the optimal administration dose and imaging time windows. Further validation has been performed in swine minimally invasive surgical models using the da Vinci Surgical Robot. We believe that the use of IT01-08 and IT05-75 in humans will improve nerve identification during surgery and reduce nerve injury, improving outcomes across all surgical specialties.

#143 - FLUORESCENT PROBES FOR INVESTIGATING MYCOBACTERIUM TUBERCULOSIS

Prof. Kimberly Beatty – Oregon Health & Science University

Dr. Samantha Levine - Oregon Health & Science University

Research in the Beatty group is focused on the development of chemical tools to understand the molecular basis of human diseases. In the area of bacterial pathogenesis, we are creating novel chemical tools for studying *Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis (TB). These small molecule probes are being used to discover new TB biomarkers and enzymes implicated in latent and active TB infections. We have used new fluorogenic probes to illuminate the activity of two types of hydrolases: sulfatases (*PNAS* 2013) and esterases (*ChemBioChem* 2014 & 2015 and *ACS Chem. Biol.*, 2016). Fluorogenic probes can be used for tracking enzyme activities because they become fluorescent in response to enzymatic hydrolysis. In 2016, we used new far-red probes to detect enzyme regulation in dormant and active Mtb. Our assay platform revealed that there is dynamic regulation of Mtb esterases and lipases during dormancy, reactivation, and active growth (*ACS Infect. Dis.*, 2016). This makes these enzymes powerful targets for classifying Mtb infections. In our most recent work, my team is synthesizing new probes for investigating enzyme regulation and drug susceptibility in Mtb. Specifically, these probes will be used to identify the targets of β-lactam antibiotics.

#136 - ACTIVITY-BASED AND KINETIC PROBES FOR CHARACTERIZATION OF BILE ACID METABOLISM IN THE GUT MICROBIOME

Dr. Kristoffer Brandvold - Biological Sciences Division, Pacific Northwest National Laboratory

Ms. Regan Volk - Pacific Northwest National Laboratory

Ms. Agne Nixon - Biological Sciences Division, Pacific Northwest National Laboratory

Ms. Jacqueline Weaver - Pacific Northwest National Laboratory

Mr. Bryan Killinger - Pacific Northwest National Laboratory

Dr. Aaron Wright - Biological Sciences Division, Pacific Northwest National Laboratory; The Gene and Linda Voiland School of Chemical Engineering and Bioengineering, Washington State University

The gut microbiota has a major impact on the metabolism of both xenobiotic and endogenous chemical agents by modulating host metabolism or by transforming the agents directly. The metabolic functions of the microbiome play crucial roles in regulation of physiological systems in the host, including the metabolic and immune systems. Characterizing the function of the gut microbiome is nearly entirely dependent on inference from metagenome sequencing, taxonomy assessments, RNAseq analyses, or on metabolomics measurements. These methods cannot assign functional activities directly to microbial cells and enzymes. Therefore, we are developing chemical biology approaches that directly measure microbial functions at the cell and enzyme scale in gut microbiomes. We have developed new activity-based and fluorescence kinetic probes to address key microbial targets including bile-modifying enzymes. Bile is an amphipathic fluid produced by the host that assists in digestion of lipophilic nutrients such as fats and hydrophobic vitamins. A key molecular component of bile is bile salts, which are cholesterol-derived small molecules that are produced in the liver and are excreted into the gut from the gall bladder upon ingestion of food. In addition to acting as surfactants, bile salts are also signaling molecules that bind host receptors to regulate host physiology. De-regulation of bile signaling is associated with a variety of diseases including cholestatic liver diseases, IBDs, fatty liver diseases, and colorectal cancer. A major route through which bile signaling may be perturbed is through metabolism of bile salts by the gut microbiome, which affects how the molecules are recognized by host receptors. The gut microbiome-mediated bile salt metabolism includes redox modification of the sterol core and de-conjugation of the amino acid moiety. While methods exist to quantify the magnitude of bile salt metabolism, these methods do not provide information on the origin of the modification. Knowing who in the gut microbiome is responsible for bile salt metabolism will provide a roadmap for harnessing this activity to combat disease and increase resilience. We have developed two new classes of chemical tools for studying bile acid metabolism including simple fluorescent reporters of bile salt hydrolysis, and activity-based probes for functional annotation of proteins and cell species involved in bile acid metabolism. In an effort to increase host resilience, are using our probes to reveal the mechanisms through which the gut microbiome regulates bile acid signaling. With our probes for bile acid metabolism we are now characterizing the impact of chemical, diet, fatigue and other exposures on bile metabolism and signaling, and also to understand the role of bile acids in metabolic disease.

#182 - Synthesis of MMP9 FRET Reporter for Lung Disease Assessment

Mr. Adam Brown – Concordia University

Dr. Victoria Halls – Oregon Health & Science University

Dr. Carsten Schultz - Oregon Health & Science University

Clinical need for relatively easy and cost-effective assessment of lung disease severity has been the subject of previous protease

reporter development. We developed a new variant of a soluble peptide-based FRET reporter to assess for upregulation of Matrix Metallopeptidase 9 (MMP9), an enzyme which has been shown to mediate pathogenic processes across multiple cell types. Post-synthesis of our peptide probe, we employed enzymatic assays to determine substrate specificity and thus verify the effective potential of our probe.

#106 - IDENTIFICATION OF INHIBITORS OF PROTEIN-RNA INTERACTIONS BY FLUORESCENCE INTENSITY-BASED BINDING ASSAY

Mr. Wan Gi Byun - CRI Center for Chemical Proteomics, Department of Chemistry, Seoul National University

Dr. Donghyun Lim - Department of Biophysics and Chemical Biology, Seoul National University

Prof. Seung Bum Park - CRI Center for Chemical Proteomics, Department of Chemistry, Seoul National University

Protein–RNA interactions regulate complex cellular pathways including gene expression and splicing. Although dysfunction of the interaction network can lead to serious illnesses such as cancer and neurodegenerative disease, protein–RNA interactions have been less studied as therapeutic targets compared to proteins or protein–protein interactions. Thus, it is desired that simple and reliable protein–RNA binding assay should be developed for the discovery of small molecules targeting the interactions. Herein, we constructed fluorescence intensity-based protein–RNA binding assays using several environment-sensitive organic fluorophores. The oncogenic interaction between Lin28 and the *let-7* microRNA can be detected using TAMRA-conjugated *let-7* probe. The fluorescence intensity of the probe increased upon specific binding with Lin28A. We performed the assay in high-throughput manner, and a pyrazolyl thiazolidinedione-type compound, **KCB170522**, was identified as hits. **KCB170522** disrupted the Lin28–*let-7* interaction in a dose-dependent manner, as validated by electrophoretic mobility shift assay. This new inhibitor enhanced cellular levels of mature *let-7* microRNAs and reduced the expression of their oncogenic target genes in Lin28A-expressing JAR human choriocarcinoma cells. Next, we developed another fluorescence intensity-based binding assay for the interaction between immunomodulatory RNA binding protein, Roquin, and its binding partner, the constitutive decay element of TNF-α mRNA (*Tnf* CDE). The fluorescence intensity of Cy3-labeled *Tnf* CDE was enhanced upon binding with Roquin, and this phenomenon was exploited for the construction of the binding assay in a multi-well plate format. The fluorescence intensity-based binding assay can be easily set up since various dye-labeled oligonucleotides are commercially available and it can be conducted without any specialized instruments. We expect that our simple and robust screening approach will be a substantial help in identifying small molecule modulators of protein–RNA interactions.

#54 - PHOTOSENSITIZATION OF THE BLIND RETINA BY AZOBENZENE-CYCLODEXTRIN COMPLEXES

Dr. Kevin Cao - UC Berkeley

Mr. Elijah Lyons - UC Berkeley

Dr. Benjamin Smith - UC Berkeley

Mr. Jonathan Shirian - UC Berkeley

Prof. Richard Kramer - UC Berkeley

Azobenzene photoswitches functionalized with a quaternary ammonium block ion channels in neurons, thereby sensitizing the cells to light stimuli. We recently reported the ability of these dyes to restore light responses to the degenerating blind eye by imbuing retinal ganglion cells (RGCs) with photosensitivity. In order to evaluate the therapeutic potential of these photoswitches to treat blindness, we developed a cyclodextrin-based delivery system utilizing host-guest chemistry to tune and prolong the photosensitization of RGCs in the blind retina. We present here the characterization of the photoswitch-cyclodextrin complex using NMR and UV-vis spectroscopy and the evaluation of its efficacy using multielectrode array (MEA) electrophysiology and calcium imaging.

#90 - INTERROGATION OF DRUG RESISTANCE IN PROTEIN KINASES USING DEEP MUTATIONAL SCANNING AND CHEMOPROTEOMIC METHODS

Ms. Sujata Chakraborty - University of Washington - Seattle

Dr. Ethan Ahler - University of Washington - Seattle

Mr. Linglan Fang - University of Washington - Seattle

Prof. Doug Fowler - University of Washington - Seattle

Prof. Dustin Maly - University of Washington

Kinases are important drug targets in cancer therapeutics, however long-term effectiveness of these treatments is limited due to emergence of point missense mutations within kinases that de-sensitizes the target kinase to the drug. It is of great importance to understand and predict the mutational routes to drug resistance in order to improve the design of next generation kinase drugs as well as for genomics-guided medicines. We use Deep Mutational Scanning (DMS) to explore the basis of drug resistance in the non-receptor tyrosine kinase Src. Different classes of ATP-competitive kinase drugs are biased towards a distinct active site conformation in which they bind. Here, we have meticulously investigated how kinase sequence confer drug resistance. We treated a library of ~3,500 Src kinase mutants against a panel of inhibitors in a massively-parallel yeast-based assay. We envision that this will help us get important insights into drug resistance in general. We uncovered various hotspots on Src's catalytic domain where resistant mutations are shared between all tested inhibitors and we also identified unique mutations that conferred resistance to just a specific class of inhibitor. This also led us to the exploration of another regulatory interface on Src's CD that co-incided with broad inhibitor resistance.

#148 - LEPTIN ACTIVATES PKA VIA A NOVEL MECHANISM TO REGULATE KATP CHANNEL TRAFFICKING IN PANCREATIC B-CELLS

Ms. Veronica Cochrane - Oregon Health & Science University

Prof. Show-Ling Shyng - Oregon Health & Science University

The adipocyte hormone leptin has a well-established role in energy homeostasis via its actions in the central nervous system. In addition, leptin has been shown to have direct actions on peripheral tissues. In pancreatic β-cells leptin inhibits insulin secretion by increasing conductance of ATP-sensitive potassium (K_{ATP}) channels, which causes membrane hyperpolarization and reduces β-cell electrical activity. There is evidence that disrupting leptin signaling at the β-cell level may lead to excessive insulin secretion and

contribute to insulin resistance and eventually type 2 diabetes. Recent studies have shown that leptin promotes K_{ATP} channel trafficking to increase K_{ATP} channel surface density and thereby K_{ATP} conductance in β-cells. We found that leptin triggers a signaling pathway that involves potentiation of NMDA receptors, leading to an influx of calcium and activation of CaMKKβ, which then phosphorylates and activates AMPK; downstream of AMPK actin remodeling occurs in a PKA-dependent manner that allows vesicles containing K_{ATP} channels to traffic to the β-cell membrane. A knowledge gap in this pathway is whether PKA plays a permissive role or whether leptin signaling that results in AMPK activation leads to increased PKA activity for actin remodeling. Here we implement a FRET-based PKA activity reporter to show that leptin signaling does indeed increase PKA activity and that this occurs via the NMDAR-CaMKKβ-AMPK signaling axis. Moreover, we provide evidence that the ability of leptin to regulate K_{ATP} channel trafficking requires that PKA is anchored to PKA anchoring proteins (AKAPs). AKAPs are scaffolding proteins known to organize signaling complexes for localized PKA signaling. Our results suggest that leptin signaling in β-cells may be localized via a signaling complex involving AKAP to achieve spatiotemporal specificity. Additional studies into AKAP-mediated organization of leptin signaling molecules in β-cells will likely provide insight as to how leptin signaling may be disrupted in pathophysiological states such as type 2 diabetes.

#92 - A CHEMICALLY INDUCIBLE ACTIVATOR OF RAS ALLOWS FOR TEMPORAL CONTROL OF RAS SIGNALING AND REVEALS RAS ISOFORM SPECIFIC SIGNALING DYNAMICS

Ms. Emily Dieter - University of Washington

Prof. Dustin Maly - University of Washington

RAS GTPases are essential players in multiple signaling pathways such as cell proliferation, differentiation, and apoptosis. Due to their frequent mutation rate in human cancers, RAS GTPases have been well studied, yet fundamental questions about basic RAS biology still remain, stemming from the complexities of RAS signaling networks and the lack of tools available to specifically and temporally activate RAS. To combat this problem, our lab has computationally designed and developed a genetically encoded RAS rheostat, termed Chemically Inducible Activator of RAS (CIAR). Our engineered switch can be controlled through user-defined inputs, thus serving as a vital tool for understanding and controlling RAS dynamics. CIAR is composed of the catalytic domain of Son of Sevenless (SOScat), a RAS activator, whose catalytic site is gated by BCL-xL/BH3 protein-peptide interactions. The BCL-xL/BH3 interaction can be disrupted using commercially available small-molecule BCL-xL inhibitors, enabling tunable activation of endogenous RAS. By appending the hypervariable region (HVR) of different RAS isoforms, CIAR can be rapidly localized to diverse cellular environments. We generated CIAR constructs that contain a C-terminal HVR from HRAS, NRAS, KRAS4A, or KRAS4B, resulting in four CIAR switches that are localized similarly to their analogous endogenous RAS isoform. Upon activation of CIAR, changes in known downstream RAS effectors can be measured. Comparison of

phospho-ERK, a canonical member of the RAS signaling pathways, reveals distinct RAS-ERK signaling dynamics, depending on which CIAR variant was used. Currently, we are using quantitative phosphoproteomics to explore global signaling changes in order to further define differences between signaling pathways initiated by each of the RAS isoforms. Future utilization of the CIAR system will help us to continue disentangling RAS isoform signaling, resulting in a better understanding of RAS biology.

#139 - VIP TAGS FOR MULTI-TARGET IMAGING BY LIGHT AND ELECTRON MICROSCOPY

Ms. Julia Doh - Oregon Health & Science University

Ms. Savannah Tobin - Oregon Health & Science University

Dr. Miguel Macias Contreras - Oregon Health & Science University

Prof. Young Hwan Chang - Oregon Health & Science University

Prof. Kimberly Beatty - Oregon Health & Science University

Microscopy allows researchers to detect proteins in their endogenous cellular environment and thus interrogate protein structure, function, distribution, and interactions. Most proteins must be tagged by a reporter to generate contrast against the cell environment. Different microscopic methods require distinct reporters. An ideal tag would be compatible with many imaging modalities such as light and electron microscopy. We have developed sets of genetically-encoded protein tags, called Versatile Interacting Peptides (**VIP**) tags, to meet this challenge. VIP tags are specific heterodimeric coiled-coil motifs formed by two peptides. One coil serves as the tag and the other as the reporter (the probe peptide). The versatility of VIP tags is imparted by the ability to conjugate different reporters to the probe peptide, thus allowing a seamless transition between light and electron microscopy. VIPs are small (<6.5 kDa), offering a substantial size reduction from commonly used protein tags, such as fluorescent proteins. We have published two VIP tags: VIP Y/Z and VIPER. We demonstrated that VIPER enables labeling of intracellular protein targets in fixed cells, tracking of membrane receptors in live cells. VIPER is also the first instance of a genetically-encoded, nanoparticle-based correlative light and electron microscopy tag. Our recent efforts are focused on miniaturized VIP tags, called MiniVIPER, TinyVIPER and PunyVIPER. These tag pairs are designed to be self-sorting to allow for targeting multiple proteins simultaneously. We will report our progress developing these tags and new applications of this technology.

#97 - FUNCTIONAL CHARACTERIZATION OF A SUBTILISIN-LIKE SERINE PROTEASE FROM VIBRIO CHOLERAE

Mr. Daniel Dumitrescu - Yale University

Mr. Matthew Howell - Yale University

Mrs. Lauren Blankenship - Yale University

Ms. Darby Herkert - Yale University

Dr. Stavroula Hatzios - Yale University

Secreted enzymes play central roles in bacterial ecology, such as mediating nutrient acquisition, biofilm assembly, or bacterial interactions with host cells, but many of these enzymes and their functions remain unknown. We recently identified a subtilisin-like serine protease secreted by the cholera pathogen *Vibrio cholerae*, IvaP, that is active

in *V. cholerae*-infected rabbits and in human choleric stool. Following secretion, IvaP is proteolytically processed to produce a truncated enzyme with serine hydrolase activity, although the mechanism of extracellular maturation has not been defined. Here, we show that IvaP maturation requires sequential N- and C-terminal cleavage, consistent with the predicted domain structure of the enzyme. We used a catalytically inactive reporter protein to demonstrate that IvaP can be partially processed in *trans*, but intramolecular proteolysis is most likely required to generate the mature enzyme. Unlike many other subtilisin-like enzymes, the IvaP cleavage pattern involves stepwise processing of the N-terminal propeptide, which serves as both an inhibitor and substrate of the purified enzyme. We show that IvaP cleaves the intestinal carbohydrate-binding protein intelectin, which inhibits intelectin binding to *V. cholerae,* and also cleaves the chitin-degrading enzyme chitodextrinase. These results suggest that IvaP may play a multifaceted role in shaping *V. cholerae* adaptation to both intestinal and aquatic environments.

#141 - LEPTIN MODULATION OF PANCREATIC B-CELL MEMBRANE POTENTIAL THROUGH PHOSPHORYLATION OF NMDA RECEPTORS BY SRC FAMILY KINASES

Dr. Assmaa Elsheikh - Oregon Health & Science University

Ms. Veronica Cochrane - Oregon Health & Science University

Mrs. Zhongying Yang - Oregon Health & Science University

Prof. Dale Fortin - Washington State University

Prof. Show-Ling Shyng - Oregon Health & Science University

Leptin, a hormone secreted by adipocytes, is known to signal directly to pancreatic β-cells to suppress insulin secretion and thus contributing to blood glucose regulation. Leptin induces trafficking of K_{ATP} and Kv2.1 channels to the plasma membrane, which causes β-cell membrane hyperpolarization to suppress insulin secretion. We have previously shown that leptin regulates K_{ATP} and $Kv_{2.1}$ channel trafficking by potentiating the activity of the NMDA subtype of ionotropic glutamate receptors. However, the cellular mechanism by which leptin regulates NMDA receptors in β-cells is still not known.

In this study, we used electrophysiological and biochemical methods to investigate the mechanism by which leptin enhances NMDA receptor activity in rat insulinoma 832/13 cells and dispersed human β-cells. We show that leptin regulates NMDA receptor activity via activation of Src family kinases. Leptin stimulation led to an increased phosphorylation of c-Src at tyrosine 418, a marker for Src kinase activation. Pharmacological inhibition of Src kinase occluded the ability of leptin to hyperpolarize β-cell membrane potential; whereas Src kinase activators mimicked the effect of leptin. Among the different NMDA receptor subunits, NR2A has been shown to undergo tyrosine phosphorylation regulation by Src kinases. Analysis of NMDA receptor subunit expression by RT-PCR and immunoblotting show that NR2A is expressed in the β-cell membrane. Expression of a dominant-negative mutant NR2A, in which the putative Src-targeting tyrosine residues have been replaced by phenylalanine (Y1292F, Y1325F, Y1387F), suppressed leptin-induced β-cell

membrane hyperpolarization, but not NMDA-induced hyperpolarization. Together these data uncover a signaling pathway by which leptin directly modulates NMDA receptors in β-cells to regulate insulin secretion. Our findings may have therapeutic implications for type 2 diabetes associated with obesity, as leptin resistance is frequently observed in obesity-induced type 2 diabetes. Elucidation of the leptin signaling mechanism in β-cells may identify potential targets to overcome leptin resistance and restore insulin secretion regulation.

#85 - Functional Lipid Probes That Span Biological Membranes

Mx. Scotty Farley - Oregon Health & Science University

Mr. Hans Leier - Oregon Health & Science University

Dr. Fikadu Tafesse - Oregon Health & Science University

Dr. Carsten Schultz - Oregon Health & Science University

Lipids are a critical component of life, contributing to energy storage, compartmentalizing biochemical processes, and relaying signaling events through a cell. There are thousands of individual lipid species in a single cell, spanning several diverse classes, and differences as subtle as the presence or absence of a double bond, or a pair of carbons, can establish radically different functions for a lipid. However, despite their fundamental utility and profound diversity, tools to elucidate the functions of individual lipids remain elusive. To this end, we have synthesized a number of individual lipids modified with functional groups to allow for derivatization (terminal alkyne handle), photocrosslinking (diazirine), and temporary protection from metabolism (coumarin photocage). We synthesized a handful of lipid families (sphingosine and sphinganine to represent sphingolipids, free fatty acid to represent a broader array of glycerolipids) and versions of these probes with the diazirine at different locations on the acyl chain, in order to enable photocrosslinking at different locations within a lipid bilayer. We have evaluated the biological fidelity of these probes using a number of biochemical and cell biological techniques, including TLC, LC-MS/MS, and live-cell microscopy, and have begun to apply them to answering elusive questions in lipid cell biology. In particular, we are looking at the ways in which different lipids (sphinganine, sphingosine, and free fatty acid) contribute to membrane remodeling and modulation of cell signaling in the context of flavivirus infection. We have shown by pharmacological and genetic ablation of sphingolipid biosynthesis that flaviviral replication relies upon sphingolipid biosynthesis, but a molecular mechanism for this dependence remains elusive. We have begun to assess the localization of sphingosine and sphinganine in Zika-infected cells by confocal microscopy, and observe a clustering of these lipids around viral replication centers. In the future, we will assess the protein associations of these lipid probes to elucidate the molecular events which may help to explain the dependence of Zika, and other flaviviruses, on sphingolipid biosynthesis.

#103 - DEVELOPMENT OF A PRODRUG STRATEGY FOR CNS DELIVERY OF NUCLEAR RECEPTOR MODULATORS

Dr. Skylar Ferrara - Oregon Health & Science University

Prof. Tom Scanlan - Oregon Health & Science University

Neurodegenerative CNS diseases remain challenging to target therapeutically due to the restriction of drug-like molecules from passing through the blood-brain barrier. Nuclear receptors are important drug targets in the CNS, however, many of their modulators contain polar functional groups like carboxylates for target engagement and thus are inhibited from crossing the blood-brain barrier. Additionally, isosteric replacement of these groups can improve brain penetration, but adverse peripheral effects mire their advancement in the clinic. Herein is described the development of a prodrug strategy which can successfully deliver more potent modulator to the brain while attenuating peripheral exposure by conversion of the parent carboxylic acid-containing drugs into amides. These amide prodrugs not only improve blood-brain barrier passage, but are substrates for a brain-residing amidase called fatty acid amide hydrolase (FAAH) while remaining masked in the periphery. FAAH cleavage of the prodrugs increases brain exposure and brain-to-serum ratios of the parent drugs up to ~100-fold compared to administration of the corresponding parent drug. Physicochemical properties, FAAH substrate validation, and comparisons of CNS vs peripheral drug action will be presented. Our results indicate this strategy can be extended to a variety of relevant carboxylic acid-containing drug structures.

#144 - UBIREAL: A HIGH-THROUGHPUT ASSAY FOR MONITORING ACTIVITIES OF UBIQUITIN-TARGETED BACTERIAL EFFECTORS IN REAL TIME

Mr. Tyler Franklin - Oregon Health & Science University

Prof. Jonathan Pruneda - Oregon Health & Science University

Ubiquitin is a post-translational modification that regulates virtually every cellular process. The ubiquitin code is regulated by E1, E2, and E3 enzyme families that generate ubiquitin signals and by deubiquitinating enzymes that remove ubiquitin signals. Dysregulation of the ubiquitin cycle is implicated in several cancers, neurodegenerative diseases, and autoimmune conditions. Additionally, pathogenic bacteria have evolved host-mimicking ubiquitin-targeted effector proteins that can disrupt proper host signaling, for example by blocking ubiquitin-mediated innate immune signaling during infection. A major bottleneck in the development and validation of small molecule modulators of the ubiquitin system is the availability of a suitable high-throughput assay for enzyme activity. We present a new assay, which we term UbiReal, that uses fluorescence polarization to monitor all stages of Ub conjugation and deconjugation in real time. We demonstrate the use of UbiReal in 1) drug screening by characterizing a known E1 inhibitor, 2) measuring enzyme specificities, and 3) monitoring the conjugation of complex Ub species by E3 enzymes and subsequent deconjugation by deubiquitinating enzymes. UbiReal has the potential to replace current methods that are both qualitative and cumbersome, as we demonstrate in our biochemical characterization of ubiquitin-targeted bacterial effectors.

Odd numbered posters present in Poster Session 1, December 13
Even numbered posters present in Poster Session 2, December 14
Ordered alphabetically by first author, presenter in bold

#71 - FLUORESCENT PHOTOCAGES FOR SPATIAL AND TEMPORAL CONTROL OF SIGNALING LIPIDS IN CELLS

Dr. Pankaj Gaur - Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences

Dr. Oleksandr A Kucherak - Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences

Dr. Yulia Ermakova - European Molecular Biology Laboratory (EMBL)

Dr. Volodymyr V Shvadchak - Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences

Dr. Dmytro A Yushchenko - Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences

Lipids serve not only as building blocks of cell membranes, but are also important components of the cell signal transduction machinery. Signaling lipids function predominantly through their interaction with proteins and display multiple roles depending on their concentration and localization. Despite their clear importance, biology of signalling lipids has not been studied as deeply as the biology of proteins or nucleic acids. This gap is partially due to the restricted choice of tools and techniques for lipid visualization and manipulation of lipid levels in living cells. Studies of lipid signaling require tools to achieve 1) selective delivery of signaling lipids at specific subcellular sites, 2) quantification of delivered lipids amount, and 3) easy control of lipid' activity.

Here, at the Institute of Organic Chemistry and Biochemistry (IOCB), we work on the development of new photolabile caging group and photoswitchable molecules to create lipids-based chemical tools bearing these important properties. Recently, we described the new fluorescent photocages for the spatial and temporal control on signaling lipid's activity in cells. The tools were used to investigate the influence of signaling lipids on intracellular Ca^{2+} level (Pankaj Gaur et al. *Chem. Commun*., 2019, DOI: 10.1039/C9CC05602E). By utilizing these photocages, studies are under progress to achieve the reversible control on lipid's activity along with spatiotemporal control, and to monitor the influence of signaling lipids on cytosolic Ca^{2+} level and insulin release from β-cells.

#80 - COMPUTATION AND LIBRARY METHODS TO IMPROVE GENETIC CODE EXPANSION FOR 3-NITROTYROSINE

Ms. Ilana Gottfried-Lee - Oregon State University

Ms. Jenna Beyer - Oregon State University

Dr. Parisa Hosseinzadeh - University of Washington - Seattle

Mr. Phil Zhu - Oregon State University

Ms. Elise Van Fossen - Oregon State University

Dr. Richard Cooley - Oregon State University

Dr. Ryan Mehl - Oregon State University

Genetic code expansion (GCE) is a tool used to site specifically encode noncanonical amino acids (ncAAs) into recombinant proteins. This technology depends on engineering orthogonal tRNA/amino acyl tRNA synthetase (aaRS) pairs that incorporate ncAAs at amber (UAG) stop codons. During translation, these ncAA suppression systems compete with endogenous Release Factor 1 (RF1, the protein responsible for terminating translation at UAG codons), causing prematurely truncated peptide to build up inside the cell. This has led to a push towards the use of "genomically recoded" RF1

knockout *E. coli* strains for GCE. These strains prevent truncation but also present a new problem: in the absence of RF1, UAG codons can be efficiently suppressed by endogenous glutamine-tRNAs, resulting in both ncAA and glutamine incorporation at UAG codons . This work presents a method to solve this problem of heterogenous protein production by using computational biology to create improved aaRS/tRNA pairs. Specifically, we use computational approaches to guide aaRS library design, using a 2nd generation 3-nitrotyrosine system as a starting point. Rosetta software identified two residues in a 2nd generation aaRS for saturation mutagenesis and directed evolution. A 3rd generation aaRS was selected from this library which outperforms its predecessor and overcomes misincorporation in RF1 knockout strains. These results show that more efficient GCE aaRS/tRNA pairs will be critical for future use of RF1 knockout strains for GCE applictions.

#108 - FROM THE PLASMA MEMBRANE TO TANGLED DNA WEBS: A ROADWAY TO TRACK, INTERROGATE AND EMPLOY SPATIALLY LOCALIZED NEUTROPHIL PROTEASE ACTIVITIES.

Mr. Matteo Guerra - European Molecular Biology Laboratory (EMBL)

Mr. Dario Frey - Translational Lung Research Center, Heidelberg

Dr. Matthias Hagner - Translational Lung Research Center, Heidelberg

Ms. Luisa Tengler - European Molecular Biology Laboratory (EMBL)

Prof. Marcus Mall - Charite´ - Universitätsmedizin Berlin

Dr. Carsten Schultz - Oregon Health & Science University

Small-airways mucus obstruction and irreversible neutrophil-driven inflammation promote extensive bronchiectasis in lung diseases such as cystic fibrosis (CF) and chronic obstructive pulmonary diseases (COPD).

To enter the airway lumen, neutrophils orchestrate the secretion and action of their proteases, namely cathepsin G (CG), neutrophil elastase (NE) and proteinase 3 (PR3). The released neutrophil serine proteases (NSPs) contribute to the innate immunity by harming pathogens and fine-tuning inflammation. Secreted NSPs are usually counteracted by endogenous antiproteases. However, the delicate balance between these two components is utterly missing in chronic inflammation. Strikingly, NSPs greedily associate to the surface of the secreting neutrophil, to the myriad of extracellular vesicles filling the airway fluid and to the tangled webs made of neutrophil extracellular traps (NETs). When fastened to such structures, NSPs are inaccessible to antiproteases and provoke damage to the connective tissue. As a result, more proinflammatory stimuli are released and the outcome is a vicious circle leading to non-resolving airway neutrophilia.

In order to expand our palette of fluorescent tools and propose an alternative drug target and inflammatory biomarker, we developed of a new series of FRET reporters, which revealed high cathepsin G activity in CF and COPD airways. We were also moved by the demand of novel advanced diagnostic technologies to examine sputum samples for the rapid characterization of new bio-indicators, the evaluation of anti-inflammatory treatments and the description

of cellular subsets in a personalized manner and on a large cohort basis. Therefore, we established a new assay based on the combination of a spatially localized FRET probe and flow cytometry and proved it to be a valuable diagnostic technology applicable in basic and translational biomedical contexts. The simplicity and throughput of the new method opened the doors to new biomedically relevant projects: 1) To identify new inflammatory markers, we questioned which are the discriminants and common traits of inflammation in CF and COPD airways. By carrying out a comprehensive characterization of sputum samples via analysis of localized protease activities, cytokines and antiprotease levels, microbiome composition and dynamics of cellular populations we found that COPD airways appear to be characterized by a situation of less severe inflammation featuring elevated but not uttermost marker levels. Nonetheless, high membrane-bound protease activity occurs independently of the disease-status, suggesting this new trait as an early-inflammation biomarker. 2) We wondered if CF and COPD exosomes carry active NE on their surface and how to measure such activity at single-nanoparticle level. Therefore, we developed a cytometric assay to monitor protease activity on human sputum particles as small as 100nm in diameter.

Finally, we synthesized and characterized small-molecule probes which tether to DNA thanks to their DNA-minor-groove-binder moiety. The reporters are able to detect and quantify CG and NE activity on NETs, making them valuable tools to study the eclectic effect these proteases have when embedded in such webs: from the activation of dormant cancer cells to the destruction of epithelial barriers in chronic inflammation.

#147 - 7-DEAZAGUANINE BASED MODIFICATIONS OF DNA

Mr. Andrew Gustafson - Department of Chemistry, Portland State University

Dr. Peter Dedon - Deparment of Biological Engineering, Massachusetts Institute of Technology

Dr. Manal Swairjo - Department of Chemistry and Biochemistry, San Diego State University

Dr. Valérie de Crécy-Lagard - Department of Microbiology and Cell Science, University of Florida

Dr. Dirk Iwata-Reuyl - Department of Chemistry, Portland State University

The diversity of post-transcriptional nucleotide modifications identified in RNA is in sharp contrast to the relatively low number of similar modifications found in DNA. The over 130 known modifications of RNA are often chemically complex and are frequently important in optimizing translation. But only 20 known DNA modifications have been discovered to date, which are frequently just simple variations of the canonical bases.

Here we report the discovery of a DNA restriction-modification (RM) system that is linked to the 7-deazaguanine based tRNA modification pathways of queosine and archaeosine. In some bacteria, certain genes of the newly identified *dpd* cluster are responsible for the formation of two previously undescribed 7-deazaguanine based DNA modifications: 2'-deoxy-7-amido-7-deazaguanosine (dADG) and 2'-deoxy-7-cyano-7-deazaguanosine (dPreQ$_0$), while the remaining genes in the cluster are implicated to be a DNA restriction system which is sensitive to these newly discovered

modifications. Interestingly, this is a remarkable example of cross-talk between RNA and DNA processing and is the only known DNA modification system that replaces a genetically encoded base with a modified base instead of directly modifying the DNA itself.

We present here the elucidation and preliminary characterization of the enzymes involved in the modification component of this RM system, specifically those responsible for the formation of $dPreQ_0$ and dADG in DNA.

#105 - LABEL-FREE TARGET IDENTIFICATION REVEALS PROTEIN B-MEDIATED AUTOPHAGIC CELL DEATH BY MARINE NATURAL PRODUCT CALLYSPONGIOLIDE

Mr. Jaeyoung Ha - Department of Biophysics and Chemical Biology, Seoul National University

Prof. Seung Bum Park - CRI Center for Chemical Proteomics, Department of Chemistry, Seoul National University

To understand mechanism-of-action of bioactive small molecules, identifying their target proteins is the first step. In conventional approaches for target identification, chemical modification of the bioactive small molecules was prerequisite for synthesizing target identification probe. For example, affinity tags or photoreactive groups were mainly introduced to the molecules for affinity-based profiling, or cross-linking to target proteins. This process often required SAR studies to select where to introduce functional tags, and its synthetic feasibility should be investigated. However, target identification has been a major hurdle when it comes to bioactive natural products due to their limited amount in nature and structural complexity. To overcome such conventional limitations, we previously developed TS-FITGE, thermal stability shift-based fluorescence difference two-dimensional gel electrophoresis, as a label-free target identification method, which does not require chemical modification of bioactive small molecules.

A marine natural product, callyspongiolide has a complex structure, a macrocyclic lactone linked to bromophenol via dienyne linker. It was previously reported that callsypongiolide had cytotoxic effect, and we also confirmed the effect against various cancer cell lines (SH-SY5Y, MCF7, HeLa, A549, HCT116, PC3, HepG2, Jurkat) with submicromolar GI_{50} potency. The cytotoxic effect of callyspongiolide was not mediated by caspase. Cleavage of caspases and PARP was not detected when callyspongiolide was treated to the cell. To investigate how callysponigolide would kill diverse cancer cell lines, we decided to perform label-free target identification using TS-FITGE, because chemical modification of callyspongiolide would require extensive time and effort.

In TS-FITGE, proteins from vehicle-treated cell are visualized with green fluorescence, and proteins from compound-treated cell with red fluorescence. Therefore, target proteins of which thermal stability was shifted by the small molecule appeared as differentially colored, green or red spots on 2-D gel. Interestingly, TS-FITGE with callyspongiolide in HeLa, Jurkat, A549 gave distinctive results. Some protein spots gradually changed to side-by-side green and red spots as temperature increased, revealed as the same proteins (protein A, protein B, protein P, protein R, protein T) by tandem mass spectrometry.

Following TS-FITGE, CETSA (cellular thermal shift assay) and knock-down studies were performed to cross-confirm TS-FITGE results, and investigate whether each target protein candidate was functionally related to callyspongiolide. Among them, when protein B or protein R was depleted, similar cell death to callyspongiolide was observed. Besides, when callyspongiolide was treated to protein B-depleted cell, further cell death was not observed, compared to additional cell death by callyspongiolide in protein R-depleted cell, meaning that protein B shared a common pathway to cell death with callyspongiolide. Protein B was known to have an essential role in autophagic flux, and so how callyspongiolide would be associated in autophagy will be further scrutinized.

#82 - THYROID HORMONE AGONISTS PROMOTE MYELIN REPAIR IN A GENETIC MODEL OF DEMYELINATION

Dr. Meredith Hartley - Oregon Health & Science University

Ms. Margaret DeBell - Oregon Health & Science University

Dr. Ian Tagge - Oregon Health & Science University

Ms. Tania Banerji - Oregon Health & Science University

Ms. Mitra Shokat - Oregon Health & Science University

Ms. Hannah Miller - Oregon Health & Science University

Prof. Ben Emery - Oregon Health & Science University

Dr. Dennis Bourdette - Oregon Health & Science University

Prof. Tom Scanlan - Oregon Health & Science University

Multiple sclerosis is caused by inflammatory demyelination, and while there are clinical therapies that address the inflammatory component of the disease, no therapies are approved to promote repair of central nervous system (CNS) myelin. Myelin is formed by cells called oligodendrocytes, which derive from oligodendrocyte progenitor cells (OPCs). OPCs comprise ~5% of the cells in the adult brain and are available to differentiate and myelinate after myelin injury. However, in progressive MS, the ability of OPCs to differentiate is impaired and therapeutic strategies are needed to help these cells overcome this block.

We are interested in utilizing thyroid hormone action to promote myelin repair. Thyroid hormone is an important signal that induces OPC differentiation during neurodevelopment and our strategy utilizes this endogenous pathway to promote repair in adult disease. Thyromimetics are synthetic thyroid hormone agonists that avoid the adverse effects associated with hyperthyroidism through isoform specificity and selective tissue distribution. Recently, the Scanlan Lab has developed a new class of prodrug thyromimetics that improve delivery of a parent thyromimetic to the CNS.

To test the thyromimetics as agents of myelin repair, we have characterized a novel genetic model of demyelination based on genetic ablation of *Myrf*, a transcription factor critical for oligodendrocyte health. The iCKO-*Myrf* model has many advantages over the standard demyelination models used in the field including a clinical behavioral phenotype and clear phases of demyelination and repair. With thyromimetic drugs, we observed an improvement in clinical motor deficits, and an

increase in myelin measured by in vivo MRI and by tissue histology.

These studies validate the use of a thyromimetic strategy for promoting myelin repair and represent the scientific basis for clinical development.

#156 - Autocrine Signaling Factors are Essential for b-Cell Activity and Insulin Secretion

Dr. Sebastian Hauke - European Molecular Biology Laboratory

Ms. Kaya Keutler - Oregon Health & Science University

Dr. Aurélien Laguerre - Oregon Health & Science University

Dr. Dmytro Yushchenko - European Molecular Biology Laboratory

Dr. Carsten Schultz - Oregon Health & Science University

Secretion of insulin in response to extracellular stimuli such as elevated glucose levels and small molecules that act on G-protein coupled receptors (GPCRs) is the hallmark of β-cell physiology. Intercellular and inter-islet coordination are mediated by small diffusible ligands of GPCRs within the extracellular space of pancreatic islets. We exploited the well documented correlation between changes of the intracellular Ca2+ concentration ($[Ca2+]_i$ oscillations) with insulin to monitor β-cell activity and insulin secretion.

ATP has been previously found co-released with insulin to modulate and propagate β-cell activity within the islets of Langerhans. We found that extensive washing or depletion of extracellular ATP levels by recombinant apyrase reduced $[Ca2+]_i$ oscillations and insulin secretion in MIN6, 1.1B4 and mouse primary β-cells. Following extensive washing, addition of ATP or stable ATP analogues stimulated $[Ca2+]_i$ oscillations. Inhibition of cellular ecto-ATP nucleotidases increased extracellular ATP levels, along with $[Ca2+]_i$ oscillations and insulin secretion. Loss of ATP was compensated by the addition of exogenous fatty acids, which were previously identified as essential autocrine signaling factors.

Trace amines (TAs) are small aromatic metabolites that were identified as low-abundant ligands of the trace amine-associated receptor 1 (TAAR1) in the central nervous system (CNS). In the presented work, we identify TAs as essential autocrine signaling factors that maintain and regulate oscillations of the intracellular $[Ca2+]_i$ oscillations along with insulin secretion from β-cells via TAAR1. We found that the modulation of endogenous TA levels by the selective inhibition of TA biosynthetic pathways directly translated into changes of $[Ca2+]_i$ oscillations and insulin secretion. Application of aromatic amine-withdrawing β-cyclodextrin temporarily reduced $[Ca2+]_i$ oscillations. This demonstrates the essential role of TAs for β-cell activity as well as their high metabolic turnover rates. Notably, herein applied inhibitors and synthetic TAAR1 (ant-)agonists are partly approved for the therapeutic modulation of biogenic amine levels within the CNS, and hence for the treatment of common neurological disorders. According to our findings, these drugs even affect β-cell activity and insulin secretion through pancreatic TAAR1.

Our combined results suggest extracellular ATPases and monoamine oxidases are a potential drug target for modulating insulin

release. Therefore, our results contribute to a more detailed and complete understanding of the general role of autocrine signaling factors as a fundamental way of regulating β-cell activity and insulin secretion.

#73 - THE EMERGENCE OF NON-CANONICAL SPHINGOLIPIDS AS MOLECULAR REGULATORS OF UNDERLYING DISEASE MECHANISMS

Dr. Deron Herr - National University of Singapore

Sphingolipids (SPs) comprise a diverse class of lipids consisting of thousands of molecularly distinct species. Their formation begins with the condensation of serine and a fatty acyl-coA to produce the essential sphingoid base. In mammals, the substrate of this reaction is most often palmitoyl-coA, which gives rise to an 18-carbon backbone structure for all subsequent metabolites. However, other backbone structures exist in significant quantities. The vast majority of studies into the functions of SLs have disregarded these non-canonical backbones, owing largely to limitations of widely used analytical techniques. We have addressed this historical shortcoming by implementing highly sensitive mass spectroscopic technology to identify changes in the sphingolipidome in different disease states. Interestingly, we have identified significant changes that selectively affect the 16-carbon SL species in vascular cognitive impairment (VCI), chemotherapy-induced peripheral neuropathy (CIPN), and in type 2 diabetes mellitus (T2DM). Notably, these changes include alterations in sphingosine 1-phosphate (S1P), which is a well-characterized ligand for a family of 5 G protein-coupled receptors. We found that the plasma level of d16:1-S1P is decreased in VCI patients, but is increased following administration of neurotoxic platinum chemotherapeutics. In both cases, the d16:1- to d18:1-S1P ratio negatively correlated with disease, suggesting that d16:1-S1P is neuroprotective. Furthermore, we have evidence to suggest that alteration of this ratio is causative, rather than a result of the disease condition, since our GWAS analysis has identified two specific loci that regulate d16:1-S1P content. Mechanistically, we show that d16:1-S1P can antagonize the inflammatory effect that d18:1-S1P has on astrocytes. This appears to be, in part, due to the differential activities of the two S1P species toward their cognate receptors. By contrast, we have found a *positive* correlation between d16:1-S1P and T2DM. Our ongoing studies are investigating the role this plays in disease progression. Cumulatively, we provide evidence that structural variations in the sphingoid backbone of signalling lipids contributes qualitatively and quantitatively to their biological properties, and are relevant in neurologic and metabolic disease.

#119 - TOOLS FOR INVESTIGATING PANCREATIC ALPHA-CELL PHYSIOLOGY

Mrs. Julia Huey - Oregon Health & Science University

Dr. Carsten Schultz - Oregon Health & Science University

Glucagon is a critical hormone secreted from α-cells of the endocrine pancreas in response to hypoglycemia. Despite being subject to study for several decades, the mechanisms controlling glucagon secretion from pancreatic α-cells are not completely understood. Heterogeneity of the α-cell population contributes to the difficulty in understanding their underlying physiology. Using established chemical tools (caged

lipids) and a novel genetically encoded tool that allows for optical control of dimerization, it is possible to study glucagon secretion from α-cells at a single cellular level. This will allow for unprecedented investigation of various paracrine signals that are thought to play a role in modulating glucagon secretion, and will lead to a better understanding of intraislet signalling.

#26 - A DNA-BASED FLUORESCENT PROBE MAPS NOS3 ACTIVITY LEVELS WITH SUB-CELLULAR SPATIAL RESOLUTION

Mr. Maulik Jani - The University of Chicago

Mr. Junyi Zou - The University of Chicago

Dr. Aneesh Tazhe Veetil - The University of Chicago

Dr. Yamuna Krishnan - The University of Chicago

Nitric oxide synthase 3 (NOS3) produces the gasotransmitter, nitric oxide (NO) that drives critical cellular signaling pathways by S-nitrosylating target proteins. Endogenous NOS3 resides at two distinct sub-cellular locations - the plasma membrane and the trans Golgi network. However, NO generation arising from the activities of both these pools of NOS3 and its relative contribution to physiology or disease is not yet resolvable. We describe a probe technology to quantitatively map the activities of endogenous NOS3 at both sub-cellular locations in live cells. We have found that though NOS3 at the Golgi is ten-fold less active than at the plasma membrane, its activity is essential for the structural integrity of the Golgi. The newfound ability to spatially map NOS3 activity provides a platform to discover selective regulators of the distinct pools of NOS3.

#93 - MANIPULATING MICROBIAL COMMUNITIES: USING CHEMICAL TOOLS TO IDENTIFY AND INHIBIT SERINE HYDROLASES IN COMMENSAL BACTERIA

Ms. Laura Keller - Stanford University

Dr. Markus Lakemeyer - Stanford University

Mr. Will Van Treuren - Stanford University

Dr. Micah Niphakis - Lundbeck La Jolla Research Center, Inc.

Dr. Kenneth Lum - Lundbeck La Jolla Research Center, Inc.

Ms. Nhi Ngo - Lundbeck La Jolla Research Center, Inc.

Prof. Justin Sonnenburg - Stanford University

Prof. Matthew Bogyo - Stanford University

Serine hydrolases play an important role in regulating proteolysis, metabolism, and cell signaling. Fluorophosphonate (FP)-containing activity-based probes have been used to identify serine hydrolases in mammalian systems and pathogenic bacteria. We are applying these probes to study commensal bacteria found in the gut with the goal of discovering novel serine hydrolases important in both host-bacteria and community interactions. Screens of diverse panels of isolated gut commensals grown *in vitro* have revealed distinct sets of serine hydrolases that are highly divergent across these different strains. Furthermore, we have identified 30 active serine hydrolases in the commonly studied *Bacteroides thetaiotaomicron*, 2 of which are putative homologs of the human dipeptidyl peptidase 4 (DPP4). Initial efforts suggest that these enzymes may be functional homologs of this human protease. Ultimately, we aim to use chemical tools to better understand and manipulate critical signaling and metabolic pathways in bacteria as a means to treat diseases associated with dysbiosis of the microbiome.

#152 - TRACE AMINES AND TAAR1 IN DIABETES AND BEYOND - HOW UNDERSTANDING SIGNALING PATHWAYS CAN HELP PREVENT DRUG SIDE-EFFECTS

Ms. Kaya Keutler - Oregon Health & Science University

Dr. Sebastian Hauke - European Molecular Biology Laboratory, Cell Biology and Biophysics Unit

Dr. Carsten Schultz - Oregon Health & Science University

Diabetes is considered an independent risk factor for the development of cardiovascular disease (CVD) in both sexes. Impaired insulin secretion from pancreatic β-cells is one of the main causes for the onset of type 2 diabetes (T2D). Paracrine and autocrine signaling are important regulatory mechanisms of insulin release. It is unknown, however, which paracrine and autocrine signaling factors or the loss of them lead to the development of diabetes and subsequently become a risk factor for CVD.

The proposed research will focus on a novel class of small-molecule compounds, so-called trace amines (TAs), that modulate β-cell activity by binding the G-protein coupled receptor (GPCR) trace amine-associated receptor 1 (TAAR1). In preliminary experiments, we showed that in the presence of glucose, TAs stimulated insulin secretion from model β-cells (MIN6) in a concentration-dependent manner; TAAR1 antagonists blocked this effect. Additionally, modulating endogenous TA levels through the inhibition of key metabolic enzymes affected secreted insulin levels accordingly. Finally, mass spectrometry analysis demonstrated that β-cells synthesized TAs in significant amounts, mimicking the trend of secreted insulin levels. We therefore hypothesize that β-cells utilize TAs as autocrine factors to fine-tune insulin release and aim to determine the underlying signaling mechanism.

Besides TAs, pancreatic TAAR1 was also found to be activated by centrally acting drugs such as antidepressants of the tricyclic family (TCAs). These drugs are often associated with significant weight gain in patients, mostly leading to discontinuation of treatment. Proof of concept *in vivo* experiments demonstrated that TCAs increased body weight compared to control mice potentially by affecting glucose metabolism. We hypothesize that the co-activation of the β-cell TAAR1 might explain the observed weight gain side-effects of these drugs and aim to investigate the physiological role of TAAR1 in glucose metabolism in the intact animal.

#134 - A MULTI-OMICS ACTIVITY-BASED ANALYSIS OF GLYCOSIDE HYDROLASE FUNCTION IN GUT MICROBIOMES FROM VARIED FIBER DIETS

Mr. Bryan Killinger - Pacific Northwest National Laboratory

Dr. Aaron Wright - Biological Sciences Division, Pacific Northwest National Laboratory; The Gene and Linda Voiland School of Chemical Engineering and Bioengineering, Washington State University

Prof. Christopher Whidbey - Seattle University

Despite the importance of the gut microbiota to health and physiology, there are limited tools available to study biochemical function within the gut. Metagenomic and metatranscriptomic studies can help identify the genes present and expressed, but they do not identify the enzymes and organisms responsible for a given activity *in vivo*. To overcome this limitation, we have developed an approach that applies activity-based protein profiling (ABPP) to gut microbiome samples for subsequent proteomics analysis.

We used a glycoside hydrolase activity-based protein profiling approach to study the impact of varied fiber concentrations in the diet. Low and high fiber diets represent differences in the modern Western diet compared to traditional, higher fiber diets. Low fiber diets have been associated with irritable bowel disease (IBD), type II diabetes, colitis, and increased susceptibility to pathogens. Coupled with metagenomics and metabolomics, glycoside hydrolase probes were used with proteomics to determine significant alterations between fiber diets, including increased mucosal degradation. Due to the complexity of microbiome proteomics, we also developed and applied a novel and highly accurate comparative method of combining peptide-level statistics. Our multi-omics study enabled a comprehensive analysis of the microbiome's response to variations in dietary fiber that can be directly correlated with increased susceptibility to pathogens, colitis, and IBD.

#50 - Structural and Biosynthetic Elucidation of Autoinducer-3 and Novel Analogs in E. coli.

Dr. Chung Sub Kim - Yale University

Ms. Alexandra Gatsios - Yale University

Prof. Jason Crawford - Yale University

While many decades of research have been conducted on the well-known model organism, *Escherichia coli*, many facets of *E. coli* biology are still unknown. This includes a molecular understanding of the signaling systems used by *E. coli* to regulate virulence and population-level phenotypes known as quorum sensing. Our lab has defined the structure and biosynthesis of autoinducer-3 (AI-3), a metabolite of previously unknown structure reported to be involved in the pathogenesis of enterohemorrhagic E. coli (EHEC). We found that AI-3 belongs to a family of analogs derived from threonine dehydrogenase (Tdh) products and "abortive" tRNA synthetase reactions, which are observed in a variety of Gram-negative and Gram-positive bacteria. From this biosynthetic pathway, we also discovered the formation of a novel indole alkaloid. With these metabolites in hand, we can now investigate the implications of these signaling molecules on the host and broader microbiome.

#67 - Voltage-Sensitive Fluorescent Dyes for Monitoring Membrane Potential of Organelles

Mr. Pavel Klier - UC Berkeley

Dr. Parker Deal - University of California San Francisco

Ms. Liliya Gabelev Khasin - UC Berkeley

Dr. Pei Liu - Stanford University

Dr. Polina Lishko - UC Berkeley

Prof. Evan Miller - UC Berkeley

Changes in voltage across cell membranes are critical in cellular signaling processes and are extensively studied in excitable cells such as neurons. However, the role of membrane potential is poorly understood in membrane-bound organelles. This is primarily due to the lack of tools available to non-invasively study changes in membrane potential in organelles. The "gold standard" in electrical measurements of biological membranes is patch-clamp electrophysiology. In this technique, the voltage across a membrane is controlled and measured by a pipette containing an electrode. However, this method is highly invasive and impossible to perform on organelles within intact cells, thus measurements are only possible if the

organelle can be isolated from the cell. Isolating an organelle is technically challenging and sometimes impossible and removes information about its behavior in its physiological environment. Voltage sensitive fluorescent dyes enable non-invasive measurements of membrane potential. In the Miller lab, we have made a series of dyes called VoltageFluors which sense membrane potential via a Photoinduced electron Transfer (PeT) mechanism. VoltageFluors orient themselves in a membrane with an aniline group inserted into the membrane and connected to a fluorescent reporter via a molecular wire. This aniline quenches the fluorescent reporter that sits outside the membrane via an electron transfer process that is proportional to the voltage gradient across the membrane resulting in changes in dye fluorescence intensity in response to changes in membrane potential. Because electron transfer happens on a timescale of nanoseconds, VoltageFluors can sense rapid changes in membrane potential. Existing VoltageFluors stain outer plasma membranes of cells and thus are not suitable to sensing voltage changes in membrane-bound organelles unless they are modified to target these membranes. Here we present two novel approaches to targeting VoltageFluors to organelles.

In one approach, we modify an existing rhodamine-based VoltageFluor developed in our lab with an acetoxymethyl ester to allow internalization and localization to mitochondria taking advantage of the natural localization of rhodamine esters to mitochondrial. The acetoxymethyl ester will then be hydrolyzed by cellular esterases to deliver a dye that will be retained in the mitochondrial inner membrane and sense voltage via its PeT sensing mechanism. Traditionally, mitochondrial membrane potential is studied via the use of lipophilic cationic dyes which diffuse in and out of the mitochondrial matrix in proportion to the charge gradient across the inner mitochondrial membrane. However, this diffusive process is kinetically slow and thus will not capture rapid changes in membrane potential that occur on timescales of milliseconds. Our novel dye enables the sensing of rapid changes in mitochondrial membrane potential in intact cells for the first time to the best of our knowledge.

In the second approach we modify the VoltageFluor with a tetrazine quencher that allows covalent ligation coupled with a turn-on response upon reaction with a transcyclooctene group. The transcyclooctene is then targeted using a HaloTag protein. This protein can be targeted to a membrane of interest, enabling targeting of the VoltageFluor to organelle membranes with reduced background signal from untargeted dye.

#30 - Temporal Control of Nucleic Acid Structure and Function with Thermoreversible Glyoxal Caging

Mr. Steve Knutson - Emory University

Ms. Aimee Sanford - Emory University

Mr. Colin Swenson - Emory University

Ms. Megan Korn - Emory University

Ms. Brea Manuel - Emory University

Dr. Jennifer Heemstra - Emory University

External control over nucleic acid structure and function is vital to characterizing their roles in biological systems and essential for their non-canonical application in therapeutics, biosensing, nanotechnology and

biocomputing. While several methods have been developed for this purpose, these techniques are largely limited to short chemically synthesized oligonucleotides, and can typically only label unmodified RNA or DNA separately. Here we show that glyoxal, a well-characterized compound that covalently attaches to the Watson-Crick-Franklin face of several nucleobases, can be utilized to rapidly and reversibly cage virtually any nucleic acid scaffold. We show that glyoxal caging can be easily installed in mild and scalable conditions, potently disrupting nucleic acid structure and function. We also extensively characterize decaging kinetics in a variety of conditions, illustrating rapid and facile removal of glyoxal caging adducts through heat and mild alkaline conditions, enabling tunable environmental control over nucleic acid assembly and activity. We first demonstrate our caging method with several DNA and RNA constructs, exhibiting tight control over structural assembly, small molecule recognition, catalysis, and protein binding. We also further illustrate the wider versatility of this technique by caging two synthetic xenonucleic acid derivatives, threose nucleic acid (TNA) and peptide nucleic acids (PNA), displaying tunable regulation of anti-sense hybridization. Lastly, we show glyoxal caging on larger nucleic acid constructs, including mRNA and whole plasmids, allowing for reversible control over gene expression. Together, glyoxalation is a straightforward and cost effective method for potent and reversible caging of virtually any nucleic acid target, offering a powerful new tool in studying natural nucleic acids in biological settings as well as employing them toward useful non-canonical roles in nanotechnology, biocomputing, and synthetic biology.

#170 - MINIMAL INVASIVE LABELING TO STUDY EGFR INTERNALIZATION

Ms. Ana Kojic - European Molecular Biology laboratory (EMBL), Heidelberg

Dr. Jan-Erik Hoffmann - MPI Dortmund

Dr. Carsten Schultz - Oregon Health Science University

EGFR endocytosis and recycling has been broadly studied, mostly using imaging techniques of EGFR tagged with fluorescent proteins. Although this method has been an irreplaceable tool in studying this process, advances in click chemistry and protein labeling have opened up a possibility of specific labeling using small dyes (1). Previous work done in our group has shown the influence of PI(3,4,5)P3 on EGFR endocytosis. Elevated levels of PIP3 induce EGFR endocytosis and recycling, and the mechanism by which this is achieved is not known (2). To elucidate the mechanism of PIP3 induced EGFR endocytosis we plan to produce different EGFR mutants that will be than checked for PIP3 induced internalization deficiency. For this purpose we will use caged versions of PIP3 that are membrane permeable. Caged PIP3 is not active because of a coumarin head group in the structure that can be realized after a laser pulse. This way we can control the release of PIP3, both spatially and temporally, and observe the effect on EGFR localization. These methods combined represent a potent platform for further studies of EGFR endocytosis that despite years of research has not been fully understood.

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2. Laketa V, Zarbakhsh S, Traynor-Kaplan A, Macnamara A, Subramanian D, Putyrski M, Mueller R, Nadler A, Mentel M, Saez-Rodriguez J, Pepperkok R, Schultz C (2014). PIP_3 induces the recycling of receptor tyrosine kinases. Sci Signal, 14;7(308):ra5.

#21 - Development of fluorescent probes for visualization and quantitative analysis of free zinc ion in intracellular organelles

Dr. Toshiyuki Kowada - Tohoku University

Ms. Tomomi Watanabe - Tohoku University

Ms. Rong Liu - Tohoku University

Dr. Toshitaka Matsui - Tohoku University

Dr. Shin Mizukami - Tohoku University

Zinc is an essential trace element abundantly present next to iron in the human body. While most part of Zn^{2+} strongly binds to proteins such as metalloproteins and transcriptional factors, free Zn^{2+} is also present at low concentration inside cells. To maintain intracellular Zn^{2+} concentration at a low level, metallothionein as a buffer of Zn^{2+} and the zinc transporter families regulate the concentration of Zn^{2+}. Therefore, the analysis of Zn^{2+} dynamics in each organelle would be important for understanding Zn^{2+}-mediated biological phenomena.

Fluorescence imaging with high sensitivity and excellent spatial resolution is a powerful tool for the detection of Zn^{2+} in living cells. To date, various Zn^{2+} fluorescent probes based on either small-molecule or protein have been developed, and quantitative analysis of free Zn^{2+} ion concentration ($[Zn^{2+}]_{free}$) in cells has been conducted using fluorescent protein-based FRET probes. However, the resulting $[Zn^{2+}]_{free}$ values in ER or mitochondria varies between reports probably because of the difference of the type of probes used or influence from the intraorganellar environment, such as pH variation and oxidative environment.

In this study, in order to obtain more insights of $[Zn^{2+}]_{free}$ in intracellular organelles, a protein-labeling technology was utilized to localize pH-insensitive small-molecule fluorescent probes to the target organelles. We designed and synthesized coumarin-based fluorescent probes for Zn^{2+} with a ligand that specifically binds to a tag protein. The synthesized probe showed almost no fluorescence in the absence of Zn^{2+}, and the fluorescence intensity greatly increased with the addition of Zn^{2+}. In addition, it was found that the fluorescence intensity of the probes was scarcely affected by pH change. Therefore, it is expected that the new probe would be useful for the detection of Zn^{2+} even in organelles with acidic pH environment, such as endosome, lysosome, and Golgi apparatus. Then, we performed live-cell imaging and confirmed that the probes surely work well in several subcellular compartments. Consequently, quantitative analysis of $[Zn^{2+}]_{free}$ in Golgi apparatus was achieved.

#130 - An Activity-Based Probe for C5a peptidase: Profiling of Group A Streptococcus in Complex Communities

Dr. Sankarganesh Krishnamoorthy - Biological Sciences Division, Pacific Northwest National Laboratory;

Dr. Andrea Steiger - Biological Sciences Division, Pacific Northwest National Laboratory;

Ms. Sarah Fansler - Biological Sciences Division, Pacific Northwest National Laboratory

Mr. Lucas Webber - Biological Sciences Division, Pacific Northwest National Laboratory

Dr. Aaron Wright - Biological Sciences Division, Pacific Northwest National Laboratory; The Gene and Linda Voiland School of Chemical Engineering and Bioengineering, Washington State University

A highly conserved surface bound enzyme, C5a peptidase (SCPA), has been identified as an important virulence factor in Group A streptococcus (GAS), which is responsible for several ailments such as strep throat, scarlet fever, necrotizing fasciitis, rheumatic fever, post-streptococcal glomerulonephritis and pneumonia and toxic shock syndrome. These diseases result in an estimated half million deaths annually. SCPA acts as an adhesion factor during the invasion of the host by binding integrin, fibronectin, and epithelial cells. Additionally, SCPA 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 cleavage has been identified to occur between His-67 and Lys-68 resulting in release of a heptamer. The present work describes development of a selective activity-based probe (ABP) based on its specific C5a cleaving trait that covalently binds SCPA. The ABP's selectivity for C5a peptidase, and in turn for GAS, is optimized by increasing the peptide length to imitate the known human C5a. The selectivity of SCPA-ABP for GAS is investigated using *S. pyogenes* and nonpathogenic *E. coli.* Furthermore, efforts are directed towards detection and separation of *S. pyogenes* from complex microbiomes by pairing SCPA-ABP labeling with fluorescence-activated cell sorting (FACS) and proteome analysis.

#87 - PHOTORELEASE OF 2-ARACHIDONOYLGLYCEROL IN LIVE CELLS

Dr. Aurélien Laguerre - Oregon Health & Science University

Dr. Sebastian Hauke - European Molecular Biology Laboratory, Cell Biology and Biophysics Unit

Dr. Jian Qiu - Oregon Health & Science University

Prof. Martin J. Kelly - Oregon Health & Science University

Dr. Carsten Schultz - Oregon Health & Science University

2-arachidonoylglycerol (2-AG) is a monoacylglycerol interacting with the cannabinoid receptors 1 and 2. These two $G_{i/o}$ protein coupled receptors are present in the pancreas where they play a role in the regulation of calcium oscillations and insulin release. We aim at understanding how 2-AG levels participates in cell excitability and glucose sensing mechanisms through cannabinoid receptors activation in β-cells. The chemo-physical properties of 2-AG complicate its use as a classical drug. Unstable in aqueous media, the 2-monoacylglycerol quickly isomerizes into the still active but less potent 1(3)-AG. Other strategies that aim at affecting 2-AG levels involve lipase inhibitors who can also disturb other signaling lipids and complicate the interpretation of experimental observations. We synthesized a caged 2-arachidonoylglycerol (cg2-AG) derivative allowing for the photo-release of the endocannabinoid in live cells, without genetic nor protein alterations.[1] The strategy used for the design of cg2-AG allowed for a convenient monitoring of the uncaging process. We characterized the photo-release of 2-AG after uncaging and further monitored its effect on

CB1 and associated signaling pathways in MIN6 cells.

[1] Laguerre *et al.*, Photorelease of 2-Arachidonoylglycerol in Live Cells, *J. Am. Chem. Soc.* (2019) 141, 42, 16544-16547

#111 - ACTIVITY-BASED CHEMICAL TOOLS FOR SELECTIVE IMAGING THE VIRULENCE FACTOR OF STAPHYLOCOCCUS AUREUS

Dr. Sumin Lee - Stanford University

Dr. Rakesh Bam - Stanford University

Prof. Jeremy Dahl - Stanford University

Prof. Matthew Bogyo - Stanford University

The bacterium *Staphylococcus aureus* colonizes over one-third of the population. *S. aureus* carriers are at high risk of infection and serve as an important source for the spread of *S. aureus*. Upon infection, *S. aureus* can rapidly disperse systemically, leading to life-threatening conditions such as endocarditis, arthritis, osteomyelitis, meningitis, and sepsis. *S. aureus* often forms highly robust biofilms that lead to the generation of multicellular aggregates surrounded by extracellular matrix (ECM). The biofilm acts as a barrier against antimicrobial agents and the host immune system, thereby complicating treatment. Furthermore, the overuse of unnecessary antibiotics has contributed to the rise of drug-resistant bacteria such as methicillin-resistant *S. aureus* (MRSA). MRSA is rapidly spreading, increasing the difficulty in treating bacterial infection. In most cases, the most common methods to detect and monitor *S. aureus* infection progress involve blood cultures, histology, or PCR and those methods still required to establish a diagnosis, leading to increased risk from the biopsy procedures. Therefore, new methods for visualizing the site of infection and subsequent response to treatment would greatly improve the current clinical management of *S. aureus* infection.

I aim to develop new chemical tools to monitor S. aureus infections *in vivo* by making use of small molecule activity-based probes (ABPs) that specifically target a family of serine hydrolase enzymes which is one of the largest secreted toxins in *S. aureus*. Previously reported new serine hydrolase of *S. aureus*, FphB as a surface-exposed serine hydrolase, are ideal targets for anti-virulence and imaging agents. I synthesized far red-shifted fluorescent probes to determine the localization of FphB on the surface of *S. aureus* to address the essential functions of the new virulence factor by achieving high spatial information with single-molecule spatial resolution. Furthermore, I attempted to prepare ultrasound contrast therapeutic agents for the selective detection of FphB at sites of infection sites to enable diagnosis *in vivo* and treat the infection by releasing the appended drug. The new methods would allow monitoring of location, size, and response of the infection to antibiotics which would enable better treatment strategies with improved patient outcomes.

#66 - A BIOCHEMICAL AND BIOPHYSICAL INVESTIGATION INTO THE PATHOLOGICAL GAIN-OF-FUNCTION OF NITRATED HSP90

Ms. Miranda Leek - Oregon State University

Mrs. Carrie Marean-Reardon - Oregon State University

Dr. Patrick Reardon - Oregon State University

Dr. Alvaro Estevez - Oregon State University

Dr. Maria Clara Franco - Oregon State University

Heat Shock Protein 90 (Hsp90) is a homodimeric, pro-survival molecular chaperone ubiquitously expressed in most organisms. Hsp90 activity is linked to a cycle of conformational changes regulated by co-chaperones and post-translational modifications. We showed that nitration of tyrosine (Y) 33 and 56 in Hsp90 by the oxidant peroxynitrite induces a cell type-dependent pathological gain-of-function; nitrated Hsp90 induces cell death in neurons, while supporting survival and proliferation of tumor cells. We also found that endogenously nitrated Hsp90 is present in several tumor cell types, yet it is consistently absent in normal tissues. Although the nitrated chaperone is a promising target for tumor-directed treatment, a better understanding of how nitration of Hsp90 induces a gain-of-function is crucial for effective and selective drug development. Here, we explore the hypothesis that nitration on Y33 and Y56 results in structural changes that expand Hsp90 functionality. Our results showed that peroxynitrite treatment dramatically increased the propensity of Hsp90 to form higher-order oligomers with a simultaneous increase in the percentage of monomers vs. dimers within the population. Peroxynitrite-treated Hsp90 dimer also showed an electrophoretic shift on Native-PAGE, consistent with a more extended dimeric conformation when compared to wild type Hsp90 dimer. The simultaneous substitution of Y33 and Y56 by nitration-resistant phenylalanine partially prevented the formation of higher-order oligomers and favored the dimeric structure. In addition, site-specific incorporation of nitrotyrosine at these two positions using genetic code expansion increased the formation of monomer vs. dimer. Together, these results suggest that nitration alters the structure of Hsp90 which may account for the pathological gain-of-function. Uncovering the structural changes upon nitration that induce this new activity may lead to the development of pharmaceuticals that selectively target nitrated Hsp90 function while leaving the unmodified, pro-survival heat shock chaperone unaffected. This work was supported by R01NS102479 from NINDS/NIH (to MCF).

#75 - TARGETING EWS-ATF1 IN CLEAR CELL SARCOMA OF SOFT TISSUE (CCSST)

Dr. Bingbing Li - Oregon Health & Science University

Dr. Weiliang Chen - Oregon Health & Science University

Prof. Xiangshu Xiao - Oregon Health & Science University

Clear cell sarcoma of soft tissue (CCSST) is an aggressive soft tissue sarcoma that typically develops in the lower extremity close to tendons and aponeuroses of adolescents and young adults. The 5-year survival is only 20% for metastatic disease. This disease is notorious for its insensitivity to chemotherapies and no targeted therapies exist. The hallmark of CCSST is characterized by a balanced t(12;22) (q13;q12) chromosomal translocation, which results in a fusion of the Ewing's sarcoma gene EWS with activating transcription factor 1 (ATF1) to give an oncogene EWS-ATF1. ATF1 is a member of the cAMP-responsive element binding protein (CREB) family transcription factor. EWS-ATF1 is constitutively active to drive expression of target genes that are normally controlled by CREB/ATF1. Preclinical studies have shown that CCSST cancer cells are dependent on EWS-ATF1 for survival. We recently developed a small molecule called 666-15 as the first

potent inhibitor of ATF1/CREB-mediated gene transcription. 666-15 is well-tolerated in vivo. We present here to evaluate the therapeutic potential of 666-15 as a novel targeted therapy for the deadly CCSST.

#59 - CHARACTERIZATION OF LEUPEPTIN BIOSYNTHESIS IN XENORHABDUS SPECIES

Mr. Jhe-Hao Li - Yale University

Prof. Jason Crawford - Yale University

Leupeptin is a broad-spectrum serine/cysteine protease inhibitor originally isolated from *Streptomyces* species and is commonly used for protein purification and autophagy inhibition. Although several biochemical studies have suggested an NRPS-like biosynthetic pathway for leupeptin production, the leupeptin biosynthetic genes have not been identified. We discovered that *Xenorhabdus* and *Photorhabdus*, which are entomopathogenic nematode symbionts that produce various natural products important for insect infection and microbial competitions, also produce leupeptin. A gene synteny analysis in *Xenorhabdus* species revealed a highly conserved gene cluster, and heterologous expression of this pathway successfully recapitulated the production of leupeptin and its intermediates. Further, we characterized the functions of individual genes and proved it's not NRPS as others proposed. Additionally, we discovered more than 20 different novel leupeptin analogs in heterologous expression studies and in *Xenorhabdus* extracts by synthesis, isolation and tandem mass spectrometry. Genome mining for this pathway suggests leupeptin biosynthesis in several other bacteria including several opportunistic human pathogens, and we demonstrate leupeptin production by *Chromobacterium violaceum* for the first time. We're also looking at the association of this pathway in some human pathogens to understand the possible interaction between these pathway dependent metabolites and our immune system.

#68 - REGULATION OF ELECTRICAL SYNAPSE CONNEXIN36 BY ITS INTERACTING PROTEINS

Dr. Xinbo Li - Casey Eye Institute, Oregon Health & Science University

It is well established that gap junctions between neurons are the morphological substrate of electrical synapses, and most of the electrical synapses are formed by gap junction connexin36 (Cx36). The ultrastructural, dye-coupling and electrophysiological studies indicated the presence of Cx36 in various areas of mammalian brain regions, which includes retina, olfactory bulb, cerebral cortex, hippocampus, reticular thalamic nucleus, suprachiasmatic nucleus, hypothalamus Inferior olive and brainstem. Genetic studies indicate a link between mutations in Cx36 and juvenile myoclonic epilepsy, which is a common form of idiopathic generalized epilepsy. Further, elimination of Cx36-containing neuronal gap junctions results in behavioral abnormality, sensorimotor impairments and deficits in learning and memory. In retina, Cx36 plays a key role for information processing in retinal neurons, where electrical synapses are essential for the transmission of rod and cone signals to ganglion cells.

Gap junctions are highly dynamic structures and the modulation of gap junctional coupling between neurons is proposed to underlie rapid shifts in neuronal network connectivity and the state of gap junctional coupling

exhibits fast alteration in response to neurotransmitter stimulation, which is in line with 2.5 to 3 hours half-life in Cx36. However, our current understanding of Cx36 regulation is largely unexplored. Like chemical synapses, we found that Cx36 contains an unique c-terminal SAYV PDZ domain binding motif, and Cx36 formed electrical synapses are emerging as multi-molecular composites and Cx36 associated with PDZ-domain containing proteins: zonula occludens-1 (ZO-1), zonula occludens-2 (ZO-2), the signaling proteins PDZ RhoGEF and Afadin, the E3 ubiquitin ligase LNX1 and LNX2, and the transcription factor ZONAB in retinal neurons. We also found that truncated Cx36ΔSAYV, which lacks PDZ domain binding motif that is essential for binding with the above PDZ domain containing proteins, were localized more intracellularly compared with full length Cx36, and there was less membrane fraction in Cx36ΔSAYV cells compared with full length Cx36, therefore indicating the functional importance of the Cx36 PDZ domain binding motif in the regulation of Cx36 function. By using chromatin immunoprecipitation, gene promoter EMSA assay, co-immunoprecipitation, fusion protein pulldown, peptide pulldown assay and ubiquitination assay, we found that ZONAB bound to Cx36 gene promoter region and regulated Cx36 gene expression. More importantly, LNX proteins can regulate Cx36 degradation via ubiquitination. These results indicate that Cx36 interacting proteins may regulate Cx36 gene expression at its transcription level, may play an essential role for Cx36 cellular localization, and may regulate Cx36 protein expression via ubiquitination.

#129 - CHEMICAL BIOLOGY APPROACHES TO STUDYING PLANT-MICROBE INTERACTIONS IN BIOENERGY CROPS

Dr. Vivian S. Lin - Biological Sciences Division, Pacific Northwest National Laboratory

Ms. Natalie C. Sadler - Biological Sciences Division, Pacific Northwest National Laboratory

Ms. Yuliya Farris - Biological Sciences Division, Pacific Northwest National Laboratory

Mr. Elias Zegeye - Biological Sciences Division, Pacific Northwest National Laboratory

Land availability for both food and bioenergy crops is limited; an estimated third of the Earth's arable land has been lost to erosion and pollution in the past 40 years. By better utilizing poorly productive soils, we can improve food and energy security. Specific microbes can enhance crop productivity by improving nutrient acquisition and drought tolerance in plants. However, plant-microbe interactions are highly complex and often poorly understood. We require a better understanding of plant-microbe interactions to advance our ability to grow crops and more efficiently utilize limited nutrient resources. In order to pursue these complex interactions at a mechanistic level, we propose the use of chemical biology tools for live imaging and proteomics analysis to track intercellular communication and nutrient exchange between host plants and root-associated microbes. We will also be able to analyze live samples ---at microscopic scales, compared to traditional bulk techniques that cannot account for the chemical and biological spatial heterogeneity in soil microenvironments. Currently, we are exploring model plant host-microbe systems, including the model grasses *Brachypodium distachyon* and *Setaria viridis*, and optimizing our plant growth platforms for

real-time, live root imaging under different nutrient conditions, substrates, and bacterial inoculants. In the future, we envision our approach will combine imaging, activity-based probes, and mass spectrometry techniques to investigate plant-microbe interactions under diverse nutrient and environmental conditions.

#64 - ENGINEERING PHOTOCLEAVABLE PROTEIN (PHOCL) VARIANTS WITH IMPROVED RATE AND EFFICIENCY OF DISSOCIATION

Ms. Xiaocen Lu - University of Alberta

Dr. Yurong Wen - University of Alberta

Dr. Wei Zhang - University of Alberta

Dr. M. Joanne Lemieux - University of Alberta

Dr. Robert E. Campbell - University of Alberta

The photocleavable protein (PhoCl) is a green-to-red photoconvertible fluorescent protein that, when illuminated with violet light, undergoes bond cleavage at the green chromophore. This bond cleavage produces a large “empty barrel” N-terminal fragment, and a small C-terminal “chromophore peptide” fragment, which spontaneously dissociate from each other. PhoCl has been used in growing number of chemical biology and cell physiology applications. However, a drawback of the original PhoCl1.0 variant was a slow rate of dissociation with a half-time about 500 s. This rate was much too slow to enable control of many important cell physiology processes, such as subcellular localization and protein-protein interactions.

In this study, we determined the X-ray crystal structures of the PhoCl1.0 green state at 2.1 Åresolution, the red state at 2.3 Å resolution, and the cleaved PhoCl empty barrel at 2.8 Å resolution. Structure comparison of the intact PhoCl1.0 and its cleaved barrel indicates that conformational changes in loops at the end of the barrel contribute to stabilization of the dissociated state. Guided by the X-ray structure, we used site-directed mutagenesis to generate libraries which were screened for rate and extent of dissociation. This screen was performed using a split NanoLuc reporter system. After targeting 43 potential sites and screening more than 10,000 variants, we found 2 improved versions named PhoCl2f (faster dissociation, $t_{1/2}$ of ∼40 s) and PhoCl2c (higher contrast ratio). The dissociation rate and ratio were verified in cells using our previous assay of manipulating subcellular protein localization through PhoCl-dependent cleavage of a nuclear export sequence (NES). Finally, we have explored the use PhoCl to achieve the optogenetic control of cell death pathways through the photoinduced dissociation of pro-apoptotic protein Bid to activate the downstream apoptotic protein.

#142 - ENABLING RESEARCH THROUGH PROVISION OF CHEMICAL TOOLS

Dr. Hannah Maple - Tocris (Bio-Techne)

Dr. Paul Wood - Tocris (Bio-Techne)

Dr. Andrew Burton - Tocris (Bio-Techne)

Prof. Christian Soeller - University of Exeter

Ms. Evelina Lučinskaitė - University of Exeter

Dr. Anna Meletiou - University of Exeter

Dr. Alex Kalyuzhny - Bio-Techne

Prof. Maddy Parsons - King's College

Ms. Willow Hight-Warburton - King's College

Dr. Robert Felix - Tocris (Bio-Techne)

Tocris, a Bio-Techne company, provides innovative new tools to enable researchers in all aspects of the life sciences. Commercial tool providers have always been a valuable part of the research ecosystem, and through

building collaborations and licensing we aim to provide a platform to both develop and disseminate new tools and technologies.

We have several funded PhD studentships and collaborations, working on generating chemical research tools to answer biological questions. Two case studies are presented below, as examples of developing novel reagents for imaging applications.

Fluorogenic probes for 'no-wash' cell imaging experiments

The Janelia Fluor® dyes developed by Luke Lavis' group provide a new series of rhodamine dyes that exhibit high brightness and photostability. An additional property of interest exhibited by some of these dyes is their fluorogenicity, which can be exploited to lower background signal and enable no-wash imaging experiments. We have used Janelia Fluor® 646, a red-emitting fluorogenic dye coupled to Taxol to develop a new probe for imaging the microtubule cytoskeleton. Data are presented illustrating the use of this compound in no-wash imaging experiments.

Optical tools for integrin biology

In collaboration with Professor Maddy Parsons' lab at King's College London, we have developed a new fluorescent probe for studying integrin-dependent control of keratinocyte migration. The compound is based on Janelia Fluor® 549 coupled to a dual inhibitor of α4β1/α9β1 integrins. The brightness and photostability of Janelia Fluor® 549 enabled fluorescent time lapse experiments revealing apparent internalisation of α4β1 integrin receptors upon binding to the inhibitor. Future work will involve development of a photo-caged version of the α4β1/α9β1 integrin inhibitor at Tocris, for spatial and temporal control of α4β1/α9β1 integrin function in migrating keratinocytes. (1)

1. Analysing contributions of α4 and α9 integrins to epithelial cell migration. Hight-Warburton, W., Felix, R., Burton, A., Maple, H., McGrath, J., Parsons, M. 2018, Mol. Biol. Cell, Vol. 28 (Abstract B621).

#18 - DEVELOPMENT OF PHOTOCHROMIC LIGANDS THAT ENABLE PHOTOREVERSIBLE PROTEIN LABELING

Mr. Takato Mashita - Tohoku University

Dr. Toshiyuki Kowada - Tohoku University

Mr. Hiroto Takahashi - Tohoku University

Dr. Toshitaka Matsui - Tohoku University

Dr. Shin Mizukami - Tohoku University

A protein labeling system is known as a versatile method that specifically labels a tag-fused protein of interest (POI) with a functional small-molecule. This system is also applied to artificial induction of a specific protein dimerization or translocation in a living cell. Several groups recently reported light-regulated protein labeling systems that consist of a protein labeling system and a caged compound. These new methods allowed control of POI's dimerization or localization with high spatiotemporal resolution in a living cell. Although these methods are powerful tools to reveal many biological processes caused by dimerization or translocation of specific proteins, it is difficult to apply them to repetitive control of the dimerization or translocation because of their irreversibility. To overcome this limitation, a photoreversible protein labeling system has been desired. To achieve the photoreversible system, we designed novel ligands that

reversibly bound to a tag protein on the basis on photochromism.

New photoreversible molecules capable of binding to dihydrofolate reductase from *Escherichia coli* (eDHFR), which is widely used as a tag protein, will be presented. We developed a photoswitchable compound in which some atoms of an original eDHFR ligand were replaced to obtain photoisomerization property. This compound reversibly isomerized upon UV light (394 nm) and green light (560 nm), and the compound under UV irradiation had higher affinity to eDHFR than that without irradiation. In this conference, we will present the details about our newly developed photoswitchable protein labeling system.

#55 - Application of Mass Cytometry to Study Protein Prenylation in Autophagy Compromised Cell Models

Ms. Zoe Maxwell - University of Minnesota, Twin Cities

Dr. Heather Brown - University of Minnesota, Twin Cities

Ms. Elyse Krautkramer - University of Minnesota, Twin Cities

Mr. Kiall Suazo - University of Minnesota, Twin Cities

Mr. Kevin Liu - University of Minnesota, Twin Cities

Prof. Mark Distefano - University of Minnesota, Twin Cities

Prof. Edgar Arriaga - University of Minnesota, Twin Cities

The percent of the United States population over the age of 60 is growing and is expected to more than double over the next 30 years. With a rise in the population of older individuals, the prevalence of age-related diseases is also expected to increase. Understanding the process of aging will be key in developing therapies to target cellular changes that lead to age-related diseases and could increase the healthspan of individuals. Autophagy function and the post-translational modification of prenylation have been implicated in age-related diseases, but not investigated in detail for the impact they may have on each other during the aging process. This project focuses on understanding the aging process at the cellular level in myoblast cells by studying the relationship between reduced autophagy function and prenylation. The method of mass cytometry was chosen for this project which uses antibodies or reporters tagged with stable lanthanide isotopes detected through inductively coupled plasma time of flight mass spectrometry. Mass cytometry enables the use of many reporters in one sample of cells with little detection overlap of the reporters and detection at the single cell level. We have designed a small molecule probe for prenylation that mimics an isoprenoid diphosphate with an additional alkyne group. The probe is metabolically incorporated during cell culture and attaches to proteins that are normally farnesylated or geranylgeranylated. A reporter containing a stable terbium isotope and azide group was made to attach to the prenylation probe using the copper catalyzed azide-alkyne click reaction. We have been able to use this probe and reporter system in L6 rat myoblast cells with limited non-specific binding to track changes in overall prenylation levels. To investigate changes in prenylation in relation to autophagy function, models for reduced autophagy function were generated from L6 rat myoblast cells. The cells were either treated with siRNA targeting autophagy related protein 7 (ATG7) or a stable knockout

of ATG7 using CRISPR Cas9 and validated using quantitative PCR. The probe and reporter system were applied in both cell models and resulted in a significant increase in incorporation of the probe in the cells with reduced autophagy function. These results suggest that when autophagy function is reduced there is a resulting impact on protein prenylation. To understand why the shifts in prenylation are occurring, a panel of antibodies tagged with metal isotopes will be developed to target markers in the autophagy and mevalonate isoprenoid synthesis pathway. The method described here has the potential to be applied to other cell models to investigate changes in prenylation in relation to additional aging tissue types or for models of age-related diseases associated with prenylation.

#36 - UNDERSTANDING GABAERGIC PHARMACOLOGY IN ZEBRAFISH

Prof. Matthew McCarroll - University of California San Francisco

Mr. Jack Taylor - University of California San Francisco

Mr. Douglas Meyers-Turnbull - University of California San Francisco

Prof. David Kokel - University of California San Francisco

Orchestration of animal behavior depends on the proper balance of excitatory and inhibitory neuronal signaling. GABA, the major inhibitory neurotransmitter, signals through a broad range of ionotropic receptors composed of pentameric combinations at least 19 different subunits. These receptors mediate diverse phenotypes of GABAergic drugs including sedatives, hypnotics, anxiolytics, and anesthetics. But, due to the complexity of receptor subtypes, their pharmacology and behavioral consequences are poorly understood. The purpose of this research is to identify compounds that affect GABA signaling pathways and understand their mechanisms of action. To identify such compounds, we used large-scale behavior-based screening for GABA-related phenotypes. These studies identified a panel of compounds associated with GABAA agonists, antagonists, and agonist/antagonist combinations. These compounds appear to interact with targets including GABAAR, 5HTR6, and mGluR5. Whole brain activity mapping with pERK shows these compounds produce differential patterns of activity. In addition, at least one of these compounds appears to produce anesthesia-related phenotypes in mice. Together, this work describes methods for identifying neuroactive compounds, elucidating the neurological and molecular mechanisms by which these compounds act, and provides a pathway for potential translation to mammalian systems.

#113 - CLICKABLE PHOTOCROSSLINKERS FOR TARGET IDENTIFICATION: SYNTHESIS AND APPLICATIONS

Dr. Qianli Meng - Oregon Health & Science University

Dr. Pedro Martin-Acosta - Oregon Health & Science University

Dr. Bingbing Li - Oregon Health & Science University

Prof. Xiangshu Xiao - Oregon Health & Science University

Natural and synthetic molecules represent powerful chemical tools to investigate human diseases and biology. One of the challenges in this area is to understand their binding target profiles in the native cellular environment. Target identification of bioactive compounds

remains critical in understanding their utility in biology. Recently, various chemical proteomics strategies have been developed to comprehensively reveal the targets of bioactive compounds. We have employed and developed target identification strategy using clickable photocrosslinkers. As an example, we have discovered nuclear lamins as the targets of an anti-cancer acylated pyrroloquinazoline compound using this strategy (Li *ACS Chem Biol* **2018**, *13*, 1810; and Li et al *ACS Cent. Sci.* **2018,** *4*, 1201). In this approach, the bioactive compounds are modified to contain a clickable handle and a photocrosslinking unit. A common clickable handle is an alkyne or an azide. While these clickable handles can be installed easily into the bioactive compound if an appropriate attachment point is discovered, the preparation of commonly used photocrosslinking unit diazirine is less straightforward. We and others have found that the existing method of preparation of diazirines often give inconsistent results, which significantly inhibit the target identification efforts. In this presentation, we provide our efficient new method of preparing diazirines and its utilization in synthesizing a clickable photocrosslinker based on natural product betulinic acid, which has shown a wide variety of biological activities including modulation of lamins. Putative targets of betulinic acid have been identified.

#69 - MACHINE LEARNING-BASED PLATFORM ELUCIDATES THE ACTIVITIES OF NATURAL PRODUCTS

Mr. Sigitas Mikutis - University of Cambridge

Ms. Stefanie Scheide - University of Jena

Dr. Tiago Rodrigues - Instituto de Medicina Molecular

Prof. Oliver Werz - University of Jena

Dr. Gonçalo Bernardes - University of Cambridge

Natural products (NPs) and their derivatives constitute a large fraction of presently utilized therapeutics. NP drugs differ from synthetic drugs in several ways – natural products have a greater variety in the size and shape of the molecule, tend to be richer in oxygen, employ a wide variety of mechanisms of action. With an ever-expanding arsenal of molecular tools, the mechanisms of NPs can be investigated to much greater detail than it was possible in the previous decades. However, many natural product scaffolds are difficult to access synthetically, imposing severe limitations on both the characterization and application. As a result, it is difficult to assemble NP libraries for screening purposes, thus It is crucial to be able to predict a suitable set of NPs for biological testing.

To circumvent these limitations, we have built a machine-learning based platform to predict the inhibitory capacities of natural products. The platform is built on publicly available data about known biomodulators and provides an activity prediction for 24,500 commercially available natural products and their derivatives. As a proof of concept, we have tested the platform against 5-lipoxygenase, a key enzyme in inflammatory pathways. Out of twelve natural products selected for testing, four exhibited good inhibitory activities (IC_{50} < 10 µM). One of them was found to modulate its target through a previously unknown mechanism with a pronounced tissue specificity. The machine learning-based biomodulator discovery platform thus

predicts NP biological activities with a good precision and is a powerful tool for elucidating novel mechanisms of action as well as expanding the domain of known biologically useful scaffolds.

#135 - ACTIVITY-BASED PROTEIN PROFILING OF XENOMETABOLISM IN HUMAN GUT MICROBIOMES AND CHEMICALLY EXPOSED MOUSE GUT MICROBIOMES

Mr. Carson Miller - Biological Sciences Division, Pacific Northwest National Laboratory

Dr. Aaron Wright - Biological Sciences Division, Pacific Northwest National Laboratory; The Gene and Linda Voiland School of Chemical Engineering and Bioengineering, Washington State University

Ms. Natalie C. Sadler - Biological Sciences Division, Pacific Northwest National Laboratory

Prof. Christopher Whidbey - Seattle University

We are developing chemical biology approaches to quantify xenometabolic activities at the host-gut microbiome interface. These assays will be used to delineate how exposures create metabolic susceptibilities and impact host physiology throughout human development. Using activity-based protein profiling, we can quantify phase I and II enzyme activities in the liver, lung, and intestine to determine how environmental, drug, and chemical exposures impact these activities. Principally, we are looking at the contribution of the gut microbiome to xenometabolism and the relationship between host and microbe enzyme activities. The human gut microbiota can have a major impact on the metabolism of chemical agents, either by modulating host metabolism or by transforming the agents or their metabolites directly. To accurately predict how an individual will respond to a xenobiotic exposure or drug treatment regimen, it is necessary to identify which microorganisms present in the gut interact with that specific agent and its related metabolites. However, the inter-individual diversity of gut microorganisms has complicated the development of such screening tools. For this reason, we are developing approaches to measure key metabolic activities in the gut, which are primarily hydrolysis and reduction reactions. We have revealed that taxonomically diverse populations can account for the same enzyme activity upon chemical exposures to the gut microbiota, emphasizing the need for functional analyses within the gut. By combining ABPs to probe directly for function *in situ*, we have isolated and identified functionally active microbial subpopulations from the gut microbiome and provided an estimation of functional redundancy. Using our novel approach, we can directly characterize and quantify the functional response of individual microbes and specific enzymes in the gut microbiome and host organs to xenobiotic exposures.

#140 - A TURN-ON FLUORESCENT SENSOR FOR DETECTING SULFURYLATION IN LIVE CELLS

Dr. Koushambi Mitra - The University of Texas at Dallas

Ms. Kierstin Page - The University of Texas at Dallas

Ms. Kira Mills - The University of Texas at Dallas

Ms. Jyothi Kallu - The University of Texas at Dallas

Dr. Steven Nielsen - The University of Texas at Dallas

Dr. Sheel Dodani - The University of Texas at Dallas

Utilizing inorganic sulfate and a cofactor, 3'-phosphoadenosine-5'-phosphosulfate (PAPS), human sulfotransferases (SULTs) catalyze the sulfurylation of endogenous and xenobiotic substrates and therefore, govern vital cellular processes.[1] For instance, sulfurylation of hormones, neurotransmitters or protein residues alters their functions and cellular distributions, and overall signal transduction pathways. Similarly, sulfurylation of administered drugs can drastically affect their pharmacological profiles. Thus, a perturbation of this process can contribute to various diseases ranging from cystic fibrosis and cancer.[2] However, despite the pivotal roles, endogenous sulfotransferase activity is vastly unexplored mainly due to lack of direct, continuous and real-time sensing platforms.[3]

In order to fill this technology gap, we have designed and synthesized the first turn-on fluorescent sensor, STS-3, to detect endogenous sulfotransferase activity. Computational modeling first confirmed that STS-3 is a substrate for the major phenol sulfotransferase, SULT1A1. Based on this, we next carried out *in vitro* enzymology with STS-3 and found that it exhibits a fluorescence turn-on response (ca. 20-fold), low micromolar binding affinity, and fast reaction kinetics with SULT1A1 under physiological conditions. Encouraged by these results, we used flow cytometry and multiphoton microscopy to monitor endogenous SULT1A1 activity with STS-3 in live cells for the first time. Taken together, we anticipate that these first generation chemical tools will lead to the development of high-throughput assays for direct imaging of enzyme activity in live systems, and establish new approaches for diagnostics and treatments of various related diseases.

1. M.W. Duffel. Sulfotransferases. in: C.A. McQueen (Ed.), Comprehensive Toxicology, third ed., Elsevier, Oxford, 2018, pp. 407–428, 10.18.

2. M. Negishi, L.G. Pedersen, E. Petrotchenko, S. Shevtsov, A. Gorokhov, Y. Kakuta, and L.C. Pedersen. Structure and function of sulfotransferases. *Arch. Biochem. Biophys.* 390 (2001) 149–157.

3. L. Feng, J. Ning, X. Tian, C. Wang, L. Zhang, X. Maa and Tony D. James. Fluorescent probes for bioactive detection and imaging of phase II metabolic enzymes. *Coord. Chem. Rev.* 399 (2019) 213026–-213040.

#126 - Targeting Dynamic Loops in Protein-Protein Interactions: Principles for Small Molecule Recognition

Dr. Brittany Morgan - University of Michigan

Ms. Amanda Peiffer - University of Michigan

Mr. Nick Foster - University of Michigan

Mr. Matthew Henley - University of Michigan

Dr. Clint Regan - University of Michigan

Dr. Brian Linhares - University of Michigan

Prof. Tomasz Cierpicki - University of Michigan

Prof. Anna Mapp - University of Michigan

Dynamic loops function beyond simply connecting protein structures; they control biological function by regulating protein conformational ensembles, allosteric networks, and molecular recognition. This regulation is critically important for protein-protein interactions (PPIs), where one or multiple binding sites in a single protein can specifically recognize ten to hundreds of unique binding partners. To date, the role of dynamic loops in PPIs has largely been studied through NMR-informed molecular dynamics and mutational analysis. The successful small

molecule targeting of dynamic loops would be an excellent addition to this toolbox; however, the lack of molecular recognition principles and screening strategies for dynamic and/or disordered protein regions is a significant roadblock for discovering ligands. The goal of my project is to develop specific small molecules to both study the function of dynamic loops in PPIs as well as elucidate the ligandability and molecular recognition principles that underpin the targeting of these critical substructures. To achieve this goal, we utilized transcriptional coactivators as model systems, which are excellent representatives of the challenges and opportunities of targeting dynamic loops in PPIs. We also used the covalent ligand discovery strategy of Tethering, where disulfide-based small molecules reversibly yet covalently bind to native cysteines. In particular, we targeted a cysteine residue on a dynamic loop within coactivator Med25, which is proposed to regulate both the allosteric network and the specific recognition of transcription factors at two unique binding surfaces. From the Tethering screen, we identified lead small molecules with two distinct molecular patterns that adopted either a linear or bent shape. Cheminformatics revealed that in addition to shape, several other factors were critical for recognition, including rigidity, electronics, and polar volume. Synthesis of small molecule derivatives confirmed the importance of these factors in molecular recognition and also the potential of dynamic loops to form *chiral cavities* that specifically recognize stereocenters. The binding mode and contacts of the small molecules were mapped using molecular dynamics and NMR, and independent of the molecular shape, the ligands indeed bound in a cavity formed between two dynamic loops. Despite binding at the same location, stopped flow kinetics identified that the linear- and bent-shaped ligands differentially regulated PPI enhancement, inhibition, and/or conformational state populations at both binding surfaces. We propose that the unique ligand shapes selectively bias loop conformations and subsequently the conformational ensemble and allosteric network of Med25. Future work will focus on establishing the selectively of these ligands, the generalizability of the molecular recognition principles, and the potential of targeting dynamic loops in PPIs *in cellulo*. Collectively, we developed the *first* framework that describes the ligandability of dynamic loops and the molecular recognition principles that govern their specific interactions with small molecules. We also expanded our current toolbox for studying dynamic loops to include covalent ligands, which have the potential to differentially and directly modulate PPIs and their conformation ensembles, decipher the connectivity of allosteric networks, and lead to the discovery of novel cellular and disease biology.

#100 - DEFINING SMALL-MOLECULE MODULATORS OF GPR126/ADGRG6 MEDIATING SCHWANN CELL DEVELOPMENT AND MYELINATION USING PHENOTYPIC DRUG DISCOVERY IN ZEBRAFISH

Dr. Rory Morgan - Vollum Institute, Oregon Health & Science University, Portland, OR

Prof. Kelly Monk - Vollum Institute, Oregon Health & Science University, Portland, OR

Myelin is a lipid-rich, multilayered membrane formed by specialized glial cells in the vertebrate nervous system. In the central nervous system (CNS), myelin is formed by oligodendrocytes (OLs), while in the peripheral nervous system (PNS), myelin is formed by Schwann cells (SCs). Myelin

functions as an essential insulator for rapid action potential propagation along axons and provides critical support in axonal transport and long-term survival. Demyelination (the loss of myelin) can lead to permanent neuronal damage and is a hallmark of multiple sclerosis (MS) in the CNS and several disorders of the PNS including peripheral neuropathy. The inability to remyelinate damaged axon tracts has prevented the proper treatment of myelin diseases, which is due partly to a poor understanding of the genetic and molecular mechanisms that control myelination.

Zebrafish have emerged as a premier vertebrate species to study the myelination mechanisms of OLs and SCs. A previous forward genetic screen in zebrafish identified the then orphan G protein-coupled receptor (GPCR) Gpr126 (Adgrg6) as essential for SC development and myelination. The function of Gpr126 is conserved in humans, highlighting the utility of the zebrafish model to study the function of this protein in myelination. Gpr126 belongs to a unique class of GPCRs known as adhesion GPCRs (aGPCRs). While Gpr126 is essential for SC myelination, the upstream activating mechanisms and downstream signaling pathways are still not completely understood. In recent years, the utility of zebrafish for *in vivo* chemical screening has greatly increased due to their lower cost, simpler yet conserved anatomy, and large clutches of embryos that readily absorb small molecules. Given these advantages, zebrafish represent a feasible option to identify small molecule effectors of complex biological processes such as myelination that are difficult to recapitulate *in vitro*.

We are currently screening a compound library set (711 compounds) of known GPCR ligands against *gpr126* mutant zebrafish to identify compounds that modulate Gpr126 function. For our primary screen, we are using a hypomorphic *gpr126* mutant allele (*gpr126*$^{st63/st63}$). In *gpr126*$^{st63/st63}$ mutants, myelin is reduced in the PNS. This phenotype is consistent, which has enabled the discovery of compounds that both enhance or suppress myelin defects. We have identified several compound classes capable of suppressing the *gpr126*$^{st63/st63}$ hypomorphic phenotype including aporphinoid alkaloids and triterpenoids. We are also counter-screening hit compounds against a loss-of-function *gpr126* mutant allele (*gpr126*$^{st49/st49}$) to identify compounds that may be acting via an indirect mechanism to modulate myelination. In total, the compounds identified in these screens can help to define mechanisms by which Gpr126 controls SC myelination and delineate pathways downstream of and in parallel to Gpr126. These screens can also lay groundwork for further understanding the biology of Gpr126, and importantly, have the potential to advance therapies to treat peripheral neuropathies and other nervous system disorders.

#43 - Optical Control of Sphingosine-1-Phosphate Formation and Function

Mr. Johannes Morstein - New York University

Dr. Rose Hill - UC Berkeley

Mr. Alexander Novak - New York University

Mr. Suihan Feng - University of Geneva

Mr. Derek Norman - UTHSC

Dr. Prashant Donthamsetti - UC Berkeley

Prof. James Frank - Vollum Institute, Oregon Health & Science University, Portland, OR

Dr. Takeshi Harayama - University of Geneva

Dr. Benjamin Williams - LMU

Prof. Abby Parrill - University of Memphis

Prof. Gabor Tigyi - UTHSC

Prof. Howard Riezman - University of Geneva

Prof. Ehud Isacoff - UC Berkeley

Prof. Diana Bautista - UC Berkeley

Dr. Dirk Trauner - New York University

Sphingosine-1-phosphate (S1P) plays important roles as a signaling lipid in a variety of physiological and pathophysiological processes. S1P regulates angiogenesis, cell proliferation and migration, immunity, and pain. S1P signals via a family of G Protein Coupled Receptors ($S1P_{1-5}$ receptors) as well as a number of intracellular proteins including HDAC, TRAF2, and PKC. Here we report on photoswitchable analogs of S1P and its precursor sphingosine, respectively termed PhotoS1P and PhotoSph. PhotoS1P enables optical control of $S1P_{1-5}$ receptor activity *in vitro*, shown through its ability to rapidly and reversibly control S1P-sensitive currents and S1P-evoked increases in intracellular Ca^{2+} via engagement of S1P receptors in cultured cells. We evaluated PhotoS1P *in vivo*, where it reversibly controlled S1P Receptor 3-dependent pain hypersensitivity in mice via activation of nociceptive somatosensory neurons. The pain hypersensitivity induced by PhotoS1P is comparable to that induced by S1P . PhotoS1P is uniquely suited for the study of S1P biology in cultured cells and *in vivo* because it exhibits prolonged metabolic stability compared to rapidly metabolized S1P. Using lipidomic analysis, we constructed a comprehensive metabolic map of PhotoS1P and PhotoSph. The formation these photoswitchable lipids was found to be light dependent, providing a novel tool to optically probe sphingolipid biology.

#83 - Measuring Dynamic Changes in the Labile Iron Pool in Vivo with a Reactivity-Based Probe for Positron Emission Tomography

Mr. Ryan Muir - University of California, San Francisco

Dr. Ning Zhao - University of California, San Francisco

Dr. Junnian Wei - University of California, San Francisco

Mr. Yung-Hua Wang - University of California, San Francisco

Dr. Adam Renslo - University of California, San Francisco

Dr. Michael Evans - University of California, San Francisco

Redox cycling of iron powers various enzyme functions crucial for life, making the study of iron acquisition, storage, and disposition in the whole organism a worthy topic of inquiry. However, despite its important role in biology and disease, imaging iron in animals with oxidation-state specificity remains an outstanding problem in biology and medicine. Here we report a first-generation reactivity-based probe of labile ferrous iron suitable for positron emission tomography (PET) studies in live animals. The responses of this reagent to systemic changes in labile iron disposition were revealed using iron supplementation and sequestration treatments in mice, while the potential of this approach for in vivo imaging of cancer was demonstrated using genetically and pathologically diverse mouse models, including spontaneous tumors arising in a genetically engineered model of prostate cancer driven by loss of PTEN.

#131 - Services Available at the OHSU Medicinal Chemistry Core

Dr. Aaron Nilsen - Oregon Health & Science University

Dr. Haihong Jin - Oregon Health & Science University

Dr. Jordan Devereaux - Oregon Health & Science University

Dr. Shanthi Nagarajan - Oregon Health & Science University

Dr. Victoria Halls - Oregon Health & Science University

At the OHSU Medicinal Chemistry Core we help researchers investigate interactions between small molecules and biological systems. To accomplish this the Med Chem Core provides medicinal chemistry, chemical biology and computational expertise to facilitate biologically oriented innovation.

Our services include custom organic synthesis, custom chemical biology, medicinal chemistry, large scale synthesis, peptide synthesis and computational chemistry.

Our shared instrumentation includes a Thermo LTQ Orbitrap Velos mass spectrometer mated to an Agilent 1260 Infinity II high-performance liquid chromatography (HPLC) system, an Agilent 1260 Infinity II Preparative HPLC, a Biotage Isolera Spektra automated flash purification system, a Biotage Initiator+ SP Wave microwave reactor, a Parr hydrogenator, a CEM Liberty Blue fully automated peptide synthesizer and a Labconco lyophilizer.

To learn more about our services, please visit our poster. One of our staff would be happy to answer any questions.

#133 - A robust fluorescence assay for detection of primary and secondary bile salt hydrolysis in the gut microbiome

Ms. Agne Nixon - Biological Sciences Division, Pacific Northwest National Laboratory

Dr. Kristoffer Brandvold - Biological Sciences Division, Pacific Northwest National Laboratory

Dr. Aaron Wright - Biological Sciences Division, Pacific Northwest National Laboratory; bThe Gene and Linda Voiland School of Chemical Engineering and Bioengineering, Washington State University

The composition of mammalian gut microbiome shapes the host's physiology in many ways, including through modification of endobiotic metabolites, such as bile acids. These acids and their derivatives facilitate absorption of hydrophobic nutrients, and additionally regulate host signaling pathways through binding host receptors. The host in-part determines the composition of the bile salt pool through production of the primary bile salts from cholesterol in the liver. The microbiome can also change the composition of this pool through a variety of metabolic transformations. An important microbial modification of bile salts is hydrolysis of a conjugated amino acid moiety, which can subsequently be used by microorganisms as a nutrient source. Bile salt deconjugation, which is catalyzed by bile salt hydrolase (BSH), is a key modification because it is required for all further microbial metabolism, which can include reduction and oxidation of the sterol core. Increasing evidence suggests a correlation between the bile acid composition and various diseases, such as colon cancer, obesity, and Alzheimer's disease. The therapeutic relevance of the BSH enzyme warrants a need for a sensitive and simple assay for continuous monitoring of BSH

activity. Thus, the aim our research was to develop a continuous fluorescence assay that allows for characterization of BSH activity with purified protein, cell lysates, and whole cells with both primary and secondary bile salts. To achieve this goal, we created a suite of synthetic substrates, which yield a fluorescent product upon BSH-dependent turnover. With this assay, we report for the first time an *in vivo* characterization of BSH activity on all primary and some of the most relevant secondary bile salts that are commonly associated with various human pathologies.

#125 - Secondary Structure-inspired Design of Small Molecule Modulators for the Study of Unexplored Protein-Protein Interactions

Mr. Wonwoo Park - Department of Biophysics and Chemical Biology, Seoul National University

Dr. Chanwoo Kim - CRI Center for Chemical Proteomics, Department of Chemistry, Seoul National University

Prof. Seung Bum Park - CRI Center for Chemical Proteomics, Department of Chemistry, Seoul National University

Multi-protein complexes, which are playing roles in signaling, transcription and any other physiological events, are typically composed of at least a enzyme combined with other proteins. These complexes usually form in a combinatorial sense, and hence the composition of the complex leads to the type, location or duration of biological functions. Thus, every single biological process may have its own unique relationship among not less than two proteins in a proximal contact, implying that targeting an individual component of the complex would be not the most informative approach. Rather, manipulating the interface of the multi-protein complex itself could be the way to gain easier access to the knowledge on cellular mechanism yet to be explored.

Peptide structures show a four-stage hierarchy illustrating the folding pattern of a protein. Distinct folding patterns of peptides appear at the stage of the secondary structure, where the sequential composition-dependent backbone hydrogen bonding results in the local folding of polypeptide chains, which manifests distinct structural information in the form of α-helix, β-turn or β-strand. Since proteins communicate each other by searching the secondary structures on the surface of their partner as a recognition motif, the development of modulators for protein-protein interaction (PPI) frequently involves the mimicry of secondary structure motifs, called peptidomimetics.

Each type of secondary structure motif is distinguishable in a structural sense, since they represent different 3-dimensional backbone atom alignment and hydrogen-bonding pattern. While peptidomimetic small molecules match their residue projection and backbone shape well with a certain secondary structure motif, they often failed to cover the characteristics of the other motif types. To span wide range of PPI binding modes with small molecule library containing privileged substructure, introduced here will be the development of central scaffold of peptidomimetics for three major secondary structure motifs, a-helix, b-turn and b-strand, from one single skeletal framework.

We generated 41-membered virtual scaffolds that commonly contains pyrimido-diazepine heterocycle differentiated by the third ring

with different stereochemistry and ring size. Among them, suitable candidates that display analogous backbone shape and residue projection were tuned according to synthetic accessibility and structural rigidity to afford the resultant three peptidomimetic scaffolds. Different scaffolds sharing the same skeleton can lead to large-membered peptidomimetic library creation that spans a variety of mode of action through similar synthetic pathway of scaffold construction and residue insertion.

The designed three scaffolds for α-helix, β-turn and β-strand were synthesized and were subjected to the structural elucidation including X-ray crystallography, which revealed that the scaffolds obey the respective structural criteria for secondary structure mimicry and show different levels of 3-dimensional spatial coverage, meaning that they can occupy different area on bioactive chemical space from each other. The results suggest that the computer-aided molecular design on a structural basis of peptide can provide the fidelity on the pre-defined structural features and allow the facile synthesis toward the wide range of binding modes of untapped inter-protein complex formation from a single privileged substructure through divergent manner.

#122 - Deep Mutational Scanning and Cysteine Scanning Mass Spectrometery Towards Mapping the Protein-Protein Interfaces of Multi-Domain Kinases

Mr. Zachary Potter - University of Washington

Prof. Dustin Maly - University of Washington

There are many well-documented examples of multi-domain kinases with regulatory domains that exert allosteric control over kinase activity by disrupting the alignment of key active site residues. Despite several decades of biochemical and structural characterization of Src Family Kinases (SFKs) and in particular, Src kinase, details about the structural and functional relationships between SFKs' N-terminal SH4 domain and catalytic domain remain unclear. Recent evidence from our lab (and the Fowler Lab, UW Genome Sciences) suggests that the catalytic domain of Src is blanketed in regulatory surfaces. In one example, the SH4 domain of Src contributes to the auto-inhibition of kinase activity by making physical contact with the catalytic domain at an orphan ligand binding pocket called the αF pocket. This intramolecular protein-protein interaction contributes to a closed global conformation equilibrium and diminished catalytic activity. It is unclear what role the SH4 domains of the other SFKs may play in regulation of catalytic activity, and whether or not the same putative regulatory interfaces recently discovered exist on other SFKs. To help probe this interaction more directly, and to study the intramolecular protein-protein interactions of Src and SFKs in general, we have developed a cysteine scanning method coupled with mass spectrometry. We conclude that this method could be applied to study the intramolecular allosteric regulatory mechanisms of other multi-domain kinases outside of the Src Family.

#31 - Chemistry and Biology of Unorthodox Protein Reactive Electrophiles

Prof. Thomas Poulsen - Aarhus University

Electrophilic compounds present unique opportunities for perturbing cellular systems and studies conducted over the last decade have convincingly demonstrated that

electrophiles can act with both high potency and specificity to modulate biological pathways [1]. Consequently, the interest in electrophiles as lead structures in drug discovery has also re-surfaced [2]. My laboratory has a strong interest in both structurally complex and unusual electrophiles due to the possibilities they offer as new molecular probes as well as for protein modification [3]. I will briefly present our most recent published projects including the development of so-called STEF-probes for cysteine-guided lysine-functionalization [4] and functionalized cyclopropenes that target glutathione S-transferase omega-1 in cells via a covalent mechanism [5]. The main focus will be on work that has led to the identification of novel covalent ligands for members of the fatty acid binding protein (FABP) family [6]. The FABP proteins facilitate a series of important cellular functions in metabolism and inflammation, e.g. as intracellular transporters of signaling lipids such as anandamide [7]. We discovered FABP5 as a direct cellular target of natural product-derivatives centered on a 3-pyrrolin-2-one scaffold using a chemical proteomics approach. I will present these results as well as insights into the covalent binding mechanism exploited by these compounds.

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#44 - PHOTOSWITCHABLE PROTACS ENABLE OPTICAL CONTROL OF TARGETED PROTEIN DEGRADATION

Mr. Martin Reynders - New York University

Dr. Bryan Matsuura - New York University

Ms. Marleen Berouti - New York University

Dr. Daniele Simoneschi - New York University

Dr. Antonio Marzio - New York University

Prof. Michele Pagano - New York University, HHMI

Prof. Dirk Trauner - New York University

PROTACs (PROteolysis TArgeting Chimeras) are bifunctional molecules that tag proteins for ubiquitylation by an E3 ligase complex and subsequent degradation by the proteasome. They have emerged as powerful tools to control the levels of specific cellular proteins and are on the verge of being clinically used. We now introduce photoswitchable PROTACs that can be activated with the temporal and spatial precision that light provides. These trifunctional molecules, which we named PHOTACs, consist of a ligand for an E3 ligase, a photoswitch, and a ligand for a protein of interest. We demonstrate this concept by using PHOTACs that target either BET family proteins (BRD2,3,4) or FKBP12. Our lead

compounds display little or no activity in the dark but can be reversibly activated to varying degrees with different wavelengths of light. Our modular and generalizable approach provides a method for the optical control of protein levels with photopharmacology and could lead to new types of precision therapeutics that avoid undesired systemic toxicity.

#155 - INVESTIGATION OF ALLOSTERIC COUPLING IN A GLUTAMATE TRANSPORTER HOMOLOGUE

Ms. Erika Riederer - Oregon Health & Science University

Dr. Francis Valiyaveetil - Oregon Health & Science University

Glutamate transporters harness the ionic gradients across cell membranes to carry out the concentrative uptake of glutamate. The sodium coupled Asp symporter, Glt_{Ph} is an archaeal homologue of glutamate transporters which has been extensively used to understand the transport mechanism. A critical aspect of the transport mechanism is the coupled binding of sodium and aspartate. The crystal structures of Glt_{Ph} shows that the Na^+ ions do not form direct interactions with the substrate, suggesting that the binding of Na^+ and substrate is coupled through an allosteric mechanism. Previous studies have suggested a critical role for hairpin-2 (HP2), the extracellular gate for the substrate binding site, in coupling the binding of sodium and asparate to $Glt_{Ph.}$ We have developed a fluorescence assay for monitoring HP2 movement by incorporating tryptophan and the unnatural amino acid, *p*-cyanophenylalanine into Glt_{Ph}. Our studies use the functional consequences of specific perturbations of both the Na^+ ions and substrate binding sites to reveal the intricate choreography of side chain conformational changes comprising the coupled binding of Na^+ and Asp essential for transport.

#95 - A CHEMICAL GENETICS APPROACH FOR IDENTIFYING PARP 7 TARGETS

Ms. Kelsie Rodriguez - Oregon Health & Science University

Dr. Mike Cohen - Oregon Health & Science University

Poly(ADP-ribose) polymerase 7 (PARP7), a member of the PARP family of enzymes (17 in humans), catalyzes the transfer ADP-ribose from NAD+ to target proteins, a process known as ADP-ribosylation. PARP7 is an interferon regulated gene and has been shown to be antiviral in certain contexts; however a mechanistic understanding of its cellular role has been limited by lack of identification of direct targets of PARP7. To address this limitation, we developed a chemical genetic strategy for identifying the direct targets of PARP7. This strategy was accomplished by synthesizing an orthogonal NAD+ analog that can be efficiently used by an engineered PARP7 mutant. Importantly, this analog cannot be used by any WT PARPs. Using our orthogonal NAD+ analog—engineered PARP7 pair, we identified 160 direct ADP-ribosylation targets of PARP7. By combining our chemical genetics strategy with proximity labeling using a biotin ligase-PARP7 fusion construct (a method commonly referred to as BioID) for identifying cellular interactors of PARP7, we identified several high confidence PARP7 targets. These protein targets support a role for PARP7 in the immune response pathway. Future studies will focus on understanding how ADP-ribosylation of a particular PARP7 target regulates immune signaling.

#47 - Making Proteins Cell-Permeable for Microscopy and Targeted Protein Modulation

Mr. Anselm Schneider - Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP Berlin)

Ms. Luise Franz - Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP Berlin)

Ms. Alice Baumann - Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP Berlin)

Prof. Heinrich Leonhardt - LMU Munich

Prof. M. Cristina Cardoso - TU Darmstadt

Prof. Christian Hackenberger - Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP Berlin)

Getting recombinant proteins and other biomolecules into the cellular interior of any given cell is a tremendous challenge, as biomolecules generally cannot cross cell membranes by themselves. Methods that would allow the delivery of biomolecules directly into the cytosol harbor great potential in both science and medicine.

Through the attachment of specialized, highly efficient, cationic cell-penetrating peptides, proteins can be made cell-permeable, and can pass through cell membranes independently of any active cellular transport. This "covalent transfection" is harmless for the cells and completely reversible and traceless once the cargo is inside the cell. This is not only usefuly in the delivery of therapeutically relevant peptides, but can also be applied to functional proteins as large as full-length IgG antibodies with a molecular weight of ~150 kDa. As antibodies cover the majority of the human proteome, this can allow immunostaining of many antigens in living, untransfected cells.

Because the delivery is robust, flexible and quick, it is also a useful tool in delivering a variety of functional proteins and protein conjugates into living cells. For example, multiple fluorescent nanobodies (antibody fragments) can be co-delivered into cells together, for use in dual colour live super-resolution microscopy.

Modifying the nanobodies with a UV-Crosslinker instead of a fluorophore can instead allow the light-triggered covalent capture of the antigen as well as other surrounding proteins. After a pulldown of the nanobody, captured proteins can be detected by proteomics, making this a useful tool for establishing the interactome of a given antigen.

Exchanging the fluorophore for a ligand recruiting an E3 ubiquitin ligase, instead leads to the polyubiquitination of the antigen. Eventually the protein of interest is degraded by the ubiquitin-proteasome system, allowing targeted degradation of antigens.

#84 - On the Road to Fixing Mitochondria: Discovery of Small Molecule Inhibitors of the Mitochondrial Permeability Transition Pore

Dr. Justina Sileikyte - Vollum Institute and Department of Chemical Physiology and Biochemistry, Oregon Health & Science University, Portland, OR

Dr. Marco Schiavone - CNR Neuroscience Institute and Department of Biomedical Sciences, University of Padova, Padova

Prof. Paolo Bernardi - CNR Neuroscience Institute and Department of Biomedical Sciences, University of Padova, Padova

Dr. Mike Cohen - Department of Chemical Physiology and Biochemistry, Oregon Health & Science University, Portland, OR

Dr. Michael Forte - Vollum Institute, Oregon Health & Science University, Portland, OR

Impaired mitochondrial fitness that leads to inappropriate cell death is well recognized as playing a pivotal role in a wide variety of human diseases. Studies have demonstrated that pathogenesis of ischemia-reperfusion injury, muscular dystrophies and neurodegenerative diseases share a common element – a lowered threshold for activation of the mitochondrial permeability transition pore (PTP). PTP, a high conductance channel in the inner mitochondrial membranes, opens in response to Ca^{2+} and to reactive oxygen species. It results in mitochondrial depolarization, burst in oxidative stress, impaired cellular Ca^{2+} homeostasis, diminished ATP generation and release of pro-apoptotic factors into the cytosol ultimately leading to cell death. In spite of the importance of the PTP, its potential as a drug target is currently not fully exploited. Indeed, at present our ability to treat diseases characterized by inappropriate PTP opening is restricted to the use of cyclosporine A (CsA) and its analogs, which desensitize the PTP indirectly by acting on the PTP regulator cyclophilin D, not the pore itself, and thus afford limited efficacy. Here we report the screening of NIH MLPCN ~360,000-compound chemical library with an aim to identify novel small molecules that would serve as direct and specific inhibitors of the PTP. We describe the screening strategy, the identification of hits belonging to isoxazole and benzamide scaffolds and their medicinal chemistry optimization which led to several very potent analogues. In isolated mouse liver mitochondria matrix swelling due to Ca^{2+} overload was inhibited with EC_{50} as low as 7.6 pM (1,000-fold lower than classical PTP inhibitor CsA) and the Ca^{2+} retention capacity was increased up to 15-fold (3-fold higher than CsA). Moreover, isoxazoles proved beneficial in zebrafish models of muscular dystrophies and protective in ischemia-reperfusion injury. Compared to prior art, these compounds are the best-in-class inhibitors of the PTP and a promising basis for the development of novel therapeutic agents for some of the most challenging human diseases featuring mitochondrial dysfunction. *Supported by funds from NIH and Leducq fondation.*

#76 - Development of Orally Bioavaliable Novel Multifunctional (E)-N-(2-((1H-indol-6-yl)amino)-2-oxoethyl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide (F-24) for the Treatment of Alzheimer's Disease

Mr. Yash Pal Singh - IIT BHU

Ms. Amruta Pandey - IIT BHU

Mr. Charan Tej Gullanki Naga Venkata - IIT BHU

Ms. Khushbu Priya - BHU

Dr. Prasanta Kumar Nayak - IIT BHU

Dr. Geeta Rai - BHU

Dr. Gyan Modi - IIT BHU

Alzheimer's disease (AD) is a multifactorial progressive neurodegenerative brain disorder characterized by gradual loss of neurons and synapses, particularly within the brain cholinergic system, resulting in loss of memory and other cognitive functions. It is the sixth-leading cause of death in the United States. The etiology of AD is not yet completely understood but it is evident from literature that low level of neurotransmitter especially acetylcholine, amyloid-beta (Aβ) aggregates, oxidative stress, and metals

dyshomeostasis are interdependently thought to play a central role in the neurodegeneration process.

Plant-derived natural products represent one of the major sources of therapeutic agents for a variety of diseases including neurodegenerative disorders. Several natural products including trans-4-hydroxy-3-methoxycinnamic acid (FA) and their hybrid analogs are under investigation as neuroprotective agents for AD. The poor aqueous solubility, weak interaction with the key enzymes (ChE), metabolic instability and selectivity for the target are major limitations associated with natural drugs including FA. In order to overcome these limitations, we designed and developed novel series of compounds where FA is connected to heterocyclic or substituted aromatic amines through suitable linker (acetyl). *In vitro* studies revealed that most of the synthesized compounds demonstrated moderate to potent inhibition of AChE and BChE at micromolar concentrations. Compound F24 was found to be the most potent ChE inhibitor with IC_{50} = 5.74 ± 0.13 µM for AChE and IC_{50} = 14.05 ± 0.10 µM for BChE respectively. The enzyme kinetics, studies confirmed the noncompetitive inhibition of the acetylcholinestrase in presence of F24 and mixed type inhibition in case of butrylcholinestrase. In DPPH assay F24 is able to quench free radical to a significant extent with IC_{50} = 57.35 ± 0.27 µM. Further, F24 modulates Aβ aggregation to a significant extent. Furthermore, the oral administration of F24 in mice showed low acute toxicity, and the Y-maze test indicated that this compound could improve scopolamine-induced memory deficit in mice. Collectively, these finding suggest that F24 can act as novel multifunctional lead candidate for the treatment of AD.

#128 - Intracellular Paired Agent Imaging Enables Improved Evaluation of Personalized Cancer Therapy Response

Dr. Allison Solanki - Oregon Health & Science University

Dr. Lei Wang - Oregon Health & Science University

Mr. Jesse Korber - Oregon Health & Science University

Prof. Kenneth Tichauer - Illinois Institute of Technology

Prof. Kimberley S. Samkoe - Dartmouth-Hitchcock Medical Center

Dr. Summer Gibbs - Oregon Health & Science University

Successful personalized cancer therapy continues to elude modern medicine due to rampant therapeutic resistance, leaving patients with limited curative options. Incomplete target engagement and acquired resistance (e.g., mutagenic, intracellular signaling pathway rewiring) are highly complex processes involving numerous substrates that are difficult to monitor *in vivo*, whereby standard techniques (i.e. western blot, immunohistochemistry) are invasive and static. In response, we have developed a dynamic, fluorescence-based model termed intracellular Paired-Agent Imaging (iPAI). iPAI quantifies intracellular protein target engagement using two small-molecule, cell-membrane-permeable agents: one targeted to the protein of interest and one untargeted, which accounts for non-specific therapeutic uptake. Currently, we have developed iPAI agents for evaluating the epidermal growth factor receptor (EGFR) family of proteins. Preliminary work has shown successful

synthesis and characterization of fluorescently-labeled erlotinib, an FDA approved therapeutic for non-small cell lung and pancreatic cancers that targets the tyrosine kinase binding domain. Initial validation of these probes has been conducted on a panel of mutant and wild-type EGFR expressing cell lines with various reported therapeutic sensitivities. Synthesis of additional iPAI agents targeting downstream effectors is ongoing and will allow us to visualize complex drug-target interactions and quantify their downstream signaling partners during treatment regimens. Together, we anticipate these probes will improve understanding of current limitations in personalized cancer therapy.

#121 - Function-Dependent Isolation and Characterization of Unannotated and Noncanonical Pathogenic Microbes from Microbial Communities

Dr. Andrea Steiger - Biological Sciences Division, Pacific Northwest National Laboratory

Ms. Sarah Fansler - Biological Sciences Division, Pacific Northwest National Laboratory

Dr. Sankarganesh Krishnamoorthy - Biological Sciences Division, Pacific Northwest National Laboratory

Mr. Lucas Webber - Biological Sciences Division, Pacific Northwest National Laboratory

Dr. Aaron Wright - Biological Sciences Division, Pacific Northwest National Laboratory; The Gene and Linda Voiland School of Chemical Engineering and Bioengineering, Washington State University

The only methods that currently exist for pathogen detection in complex microbial community samples rely on genomic signatures to predict pathogenicity, and they are limited in their accuracy. Along with collaborators with expertise in soil isolation and maintenance, proteomics, bioinformatics, and data engineering, we are developing and deploying chemical probes for function-dependent separation and isolation of uncultivated microbes from complex soil microbiomes based on pathogen-associated traits. We are developing a library of probes that target microbes within complex soil samples with phenotypic traits relevant to pathogenicity, such as niche finding, ability to harm the host, and self-preservation. To achieve this goal, we are designing both click-chemistry enabled activity-based probes (ABPs) that facilitate enrichment of target proteins by LC-MS based proteomics, and turn-on fluorescent probes which, upon detection of a specific pathogen-related enzyme activity, elicit a fluorescence “on” state and remain trapped within a living cell. These probes are selective for specific pathogen-associated enzyme activities including quorum sensing, type III secretion, and expression of ß-lactamase, and will ultimately allow for detection and isolation by fluorescence-activated cell sorting (FACS). Selectivity for specific pathogen-associated phenotype expression is confirmed through the use of plate reader assays, gel electrophoresis, and flow cytometry prior to application of the probes to complex bacterial communities for isolation of labeled pathogens. Using this probe-enabled strategy paired with FACS, we will isolate living, pathogenic bacteria from complex communities based on their pathogenic functions for further investigation. Additionally, the chemical probes and labeling strategies we are developing here will have broader applications that will allow for function-dependent isolation of live bacteria

of interest from a variety of complex microbiomes.

#94 - A PARP INHIBITOR SCREENING PLATFORM IN A CELLULAR CONTEXT

Dr. Sunil Sundalam - Oregon Health & Science University

Poly(ADP-ribose) polymerases (PARPs) are a family (17 in humans) of multi-domain proteins that catalyze a post-translational modification known as ADP-ribosylation. PARPs bind to nicotinamide adenine dinucleotide (NAD^+) and cleave the nicotinamide-glycosidic bond and subsequently transfer ADP-ribose to protein substrates. The biological functions of PARPs remain relatively unknown, in part due to the lack of selective small molecule inhibitors that can be used as tools for investigating their function. To facilitate the development of selective inhibitors of individual PARPs, we sought a generalizable PARP screening platform that could be used in a cellular context. We envisioned a competition-based assay using a clickable small molecule NAD^+-like probes that binds to all PARP family members. We will report our initial efforts toward this goal.

#138 - OPTICAL CONTROL OF CANNABINOID RECEPTORS

Ms. Janelle Tobias - Oregon Health & Science University

Mr. Xander Viray - Oregon Health & Science University

Ms. Gabriela Rajic - Oregon Health & Science University

Mr. Samuel Freeman - Oregon Health & Science University

Prof. James Frank - Oregon Health & Science University

The endocannabinoid (EC) system consists of cannabinoid (CB) receptors, lipophilic endocannabinoid ligands, and the enzymes controlling their bioavailability. CB1 and CB2 are inhibitory class-A G protein-coupled receptors that are expressed in immune cells (CB2) or the central nervous system and periphery (CB1). Orphan receptor GPR55 is a CB receptor that requires further investigation as to its role in the EC system. Unfortunately, no tools currently exist which allow for endogenous control of CBR signaling with subcellular precision. Genetic approaches fail to mimic acute changes in receptor activity and ligand metabolism, while traditional pharmacology is diffusion-limited and fails to recapitulate the spatial aspects of CBR activity. Photopharmacology is a powerful approach for achieving temporal manipulation of signaling pathways. Probes can be either photoswitchable (reversible) or photocaged (irreversible). By combining optically-induced tools with genetically-encoded protein tags, we can achieve spatiotemporal control within our system. Our goal is to understand the endocannabinoid system by developing optical tools to manipulate cannabinoid signaling with greater spatiotemporal control.

#53 - ENGINEERING TURN-ON FLUORESCENT PROTEIN SENSORS FOR PROBING CELLULAR CHLORIDE

Ms. Jasmine Tutol - University of Texas at Dallas

Dr. Sheel Dodani - University of Texas at Dallas

Chloride is the most abundant anion in the human body with intracellular concentrations ranging up to 70-100 mM. The transport of chloride is linked to numerous cellular

functions including cell volume, pH regulation, cell division, muscle contraction, and neuroexcitation. However, dysregulation of cellular chloride transport has been implicated in human diseases including cystic fibrosis, pancreatitis, and epilepsy suggesting that chloride could be a signal of cellular status. However, in this context, we lack a clear molecular-level picture of what chloride is actually doing. Existing quinolinium/acridinium-based dyes and yellow fluorescent protein-based sensors can be biocompatible approaches for imaging cellular chloride, but these sensors quench in the presence of chloride and/or are pH-dependent, translating into high background emission, loss of spatial and temporal resolution, thus requiring rigorous controls. A turn-on fluorescent sensing approach could be an alternative to the current state of the art but is largely underdeveloped. In this presentation, we will describe the development, characterization, and cellular applications of genetically encoded fluorescent biosensors that turn-on in the presence of chloride.

#120 - REDEFINING THE LIFETIME OF NITROTYROSINE POST-TRANSLATIONAL MODIFICATIONS IN LIVING SYSTEMS

Ms. Elise Van Fossen - Oregon State University

Mr. Phil Zhu - Oregon State University

Dr. Richard Cooley - Oregon State University

Dr. Ryan Mehl - Oregon State University

The accumulation of the nitrotyrosine (nitroTyr) post-translational modification, believed to be a stable biomarker formed under oxidative stress, is implicated in more than 50 human pathologies, including Alzheimer's disease and ALS. The site-specific incorporation of nitroTyr into proteins with genetic code expansion (GCE) has verified that a single nitroTyr modification can lead to disease pathologies, however whether or not nitrotyrosine plays a role in cellular function besides contributing to disease pathology is still uncertain. Here we present evidence suggesting that tyrosine nitration, previously thought to be irreversible, can be removed enzymatically or chemically from proteins in a biological context, suggesting that nitroTyr may play a more sophisticated role in cell biology and disease.

#153 - THE SELECTION OF FUSED VESICLES AND QUANTIFICATION OF SECRETED INSULIN IN SINGLE VESICLES BY TWO COLOR LIVE ß-CELL IMAGING

Dr. Lei Wan - Oregon Health & Science University

Labeling cargo molecules with a single fluorescence protein in dense core vesicles (DCV) of pancreatic beta cells is widely employed in the study of regulatory secretion of insulin by evanescent-field microscopy. Reliance upon a single fluorescence change nonetheless yields uncertainty when identifying a fusion event. A common issue is that the considerable number of vesicles exhibit mobility under cell culture conditions that are maintained during live imaging. Vesicular motion, e.g. newcomers or escapers, could generate sudden fluctuations in fluorescence levels that obscure the true signal from a fusion event. Here, we report a genetically-encoded, insulin-based dual-fluorescence sensor that is stably expressed in an isolated clone of MIN6 cells. This sensor specifically labels insulin-containing secretory DCV and indicates insulin secretions through synchronous, opposite changes of green and red fluorescence detected by two-color TIRF

imaging in live pancreatic beta cells. Both A-peptide sfGFP and C-peptide mCherry were secreted upon either high glucose or anti-diabetic drug stimuli, as ascertained by biochemical assay. In live imaging, A-peptide sfGFP in sensor presented as an increased brightness of green signal corresponding to vesicle with fusion pore opening. Synchronously, C-peptide mCherry in sensor presented as a rapid, quantifiable drop in red signal corresponding to insulin release at a single-vesicle resolution. Surprisingly, Imaging analysis reveals that there is significant difference of C-peptide mCherry release in single vesicles upon stimulations between high concentration glucose and anti-diabetic drug sulfonylureas. Taken together this dual-fluorescence sensor with complementary signal change will greatly enhance the selection and study of fused vesicles in insulin secretion with high-resolution live imaging.

#116 - Near Infrared Nerve-Binding Fluorophores Facilitate Buried Nerve Visualization during Laparoscopic Surgery

Dr. Lei Wang - Oregon Health & Science University

Dr. Connor Barth - Oregon Health & Science University

Mr. Antonio Montaño - Oregon Health & Science University

Dr. Alexander Antaris - Intuitive Surgical Inc.

Dr. Jonathan Sorger - Intuitive Surgical Inc.

Dr. Summer Gibbs - Oregon Health & Science University

Surgery has a prominent role in clinical medicine with over 300 million surgeries performed annually worldwide. Even with advanced surgical techniques and tools, such as high-resolution preoperative imaging and minimally invasive surgical techniques becoming routine, surgeons still rely mainly on the basic tools of anatomical knowledge and white light visualization for guidance during surgery, leaving comorbidities, such as persistent postsurgical numbness, chronic pain, and/or paralysis as a result of iatrogenic nerve injury. Nerve-sparing can be a difficult task due to patient-patient variability and the difficulty of nerve visualization in the operating room. Fluorescence-guided surgery (FGS) has the potential to revolutionize surgery by enhancing the visualization of specific tissues, such as critical nerve tissue that must be preserved in order to maintain postoperative functions. FGS offers sensitive, real-time, wide-field imaging at millimeter to centimeter depths using optical imaging of targeted fluorescent probes during surgery. However, to date, no clinically approved nerve-specific contrast agent exists. Contrast agents that fall within the near-infrared (NIR) window(650-900 nm)are particularly attractive for FGS because absorbance, scattering, and autofluorescence are all at local minima, making tissue light penetration maximal in this range. Several classes of nerve-specific small-molecule fluorophores have been studied preclinically for FGS, however, none exhibit peripheral nerve specificity and NIR fluorescence, leaving an unmet need for a viable NIR nerve contrast agent for clinical translation. A library of 64 oxazine derivatives was synthesized and characterized for optical and physicochemical properties. 42 of the novel compounds had maximum emission wavelengths that fell within the NIR imaging window. Nerve specificity screening was completed using murine models, 39 and 14 oxazine derivatives yielded positive nerve contrast following direct and systemic administration, respectively. However, only 8 compounds

demonstrated positive nerve fluorescence following both administration strategies and providing NIR emission at the same time. Several lead NIR compounds have been identified, and their nerve specificity and scalability have been confirmed in swine models, where LGW01-08 and LGW05-75 demonstrated excellent nerve specific contrast, providing clear identification and visualization of micron-scale nerve tissue buried under 2-3 mm of mixed muscle and adipose that was not possible using conventional white light alone. Importantly, NIR nerve-specific fluorophores produced nerve contrast against all key surgical tissue classes (e.g., muscle, adipose, peritoneum, and vasculature) where exposed swine iliac plexus nerves demonstrated SBRs up to ~25. The library of oxazine derivatives and screening data provide useful information in determining the pharmacophore responsible for nerve-specific uptake and retention. Furthermore, a medicinal chemistry approach was taken, enabling modification of the lead library compounds with red-shifted emission to above 800 nm while maintaining nerve specificity. These first-in-class NIR nerve-specific fluorophores demonstrated the deepest reported nerve imaging, extending nerve visualization from the tissue surface to 3 mm below mixed normal tissues. Clinical translation of these novel agents for FGS could substantially reduce comorbidities associated with surgical nerve damage.

#123 - Enzymatic Analysis of Class I HDAC complexes on Semi-synthetic Histones and Nucleosomes

Dr. Zhipeng Wang - Harvard Medical School

Acetylation, as one of the most abundant PTMs in our proteome, is regulated by two types of enzymes, Histone Acetyltransferase (HAT) and Histone Deacetylase (HDAC). Although only existing in the cell nuclei, HDAC1/2 cannot recognize nucleosome or bind DNA by itself, while previous enzymology study using histone N-terminal peptides as substrate showed no selectivity over various acetylation sites. But instead, HDAC1 binds with different protein partners serving as Co-repression complexes recruited by specific transcription factors, which regulates its deacetylation activities and biological functions. Here, we use semi-synthetic histones and nucleosomes with site-specific acetylation installed as substrates to study the enzymology in vitro. Class I HDAC complexes, including four core HDAC1 complexes known so far, LHC (CoREST), NuRD (MTA), MiDAC (MiDEAS), Sin3B (Sin3) as well as the only HDAC3 complex, NCoR (SMRT), have been investigated over different acetylation sites at histone H3K9, K14, K18, K23 and K27. We found different complexes show sharp difference in both activity over nucleosome assay but remains similarly active over histone protein assay. And different complexes have various preference over different histone sites. This provides insights into the different structures and substrate-binding model for each complex, which may be beneficial for medical applications.

#109 - An in vivo investigation into the contribution of DNA binding and cleavage to on- vs. off-target discrimination by SpCas9

Ms. Cindy Wei - University of Washington - Seattle

Prof. Dustin Maly - University of Washington - Seattle

Prof. Doug Fowler - University of Washington - Seattle

The development of CRISPR-Cas9 has been a powerful tool for genome engineering. Although Cas9 is fairly accurate for on-target (ON) loci, Cas9 suffers from off-target (OT) editing inaccuracies. High-fidelity Cas9 variants have been engineered based on *in vitro* studies to improve Cas9 targeting. However, these high-fidelity variants have problems with editing efficiency at some ON loci and also do not eliminate all OT editing activities *in vivo*. This suggests that we still do not have a full understanding of Cas9's mechanism *in vivo*; specifically, Cas9's ON vs. OT discrimination. To explore Cas9's mechanism in a cellular context, we have engineered a single-peptide, chemically-inducible Cas9 variant, ciCas9, that can be rapidly activated and rheostatically tuned with a small molecule, A115. Using ciCas9 we have been able to probe at kinetic differences in ON vs. OT editing as a possible mechanism of OT discrimination. Interestingly, we observe that overall editing kinetics at ON and OT loci are very similar. Thus, Cas9's OT discrimination mechanism must be intrinsic to the enzyme, specifically in the binding and/or cleavage of target DNA. Current experimental approaches are unable to dissect DNA binding and cleavage activities of Cas9 *in vivo*. In addition, with Cas9's predicted DNA binding affinity to be low nanomolar, most *in vivo* experiments are operating in a regime where the enzyme is at saturating levels. We are able to overcome these limitations by using ciCas9 to dissect these components in a cellular context. By titrating the amount of A115 added, we can tune the amount of available ciCas9 that can bind to DNA. This unique ability of ciCas9 coupled with high-throughput sequencing to measure indel formation and an engineered synthetic locus reporter allows us to study the occupancy of ON vs. OT loci and investigate their relative binding affinities. Finally, using a cleavage-impaired variant of ciCas9, we are able to probe at the contributions of cleavage to ON vs. OT discrimination. These studies provide *in vivo* insight into Cas9's OT discrimination mechanism through binding and cleavage competency and allow for future development of high-fidelity Cas9 variants that are optimized for *in vivo* use.

#16 - ORTHOGONAL BIOLUMINESCENT PROBES FROM HYBRID LUCIFERINS

Ms. Sierra Williams - University of California, Irvine

Mr. Zi Yao - University of California, Irvine

Prof. Jennifer Prescher - University of California, Irvine

Bioluminescence imaging is routinely used to monitor cellular interactions in real time and *in vivo*. While versatile, this technology has been largely limited to tracking one target at a time. To address this limitation, we are expanding the bioluminescence toolkit by engineering new light-emitting enzymes (luciferases) and synthetic substrates (luciferins) that can be used simultaneously (i.e., "orthogonal pairs"). These orthogonal probes comprise sterically and electronically modified luciferins that are selectively processed by mutant luciferases. Current technology only allows for monitoring two targets simultaneously. To expand this system to allow monitoring of more complex environments, we developed a new class of disubstituted luciferins that were designed to be orthogonal to existing probes. The analogs exhibited unique patterns of light output with mutant luciferases. Moreover, they were poor substrates for enzymes that were previously evolved to use monosubstituted luciferins.

These data suggested that the disubstituted analogs will be useful bioluminescent probes and facilitate multicomponent imaging studies.

#14 - EXPANDING THE BIOLUMINESCENT TOOLBOX WITH FLEXIBLE LUCIFERINS

Mr. Zi Yao - University of California, Irvine

Ms. Caroline Brennan - University of California, Irvine

Mr. Brendan Zhang - University of California, Irvine

Prof. Jeremy Mills - University of California, Irvine

Prof. Jennifer Prescher - University of California, Irvine

Bioluminescence imaging with luciferase-luciferin pairs is commonly used for monitoring biological processes *in vivo*. While popular, this technology remains limited in scope. Only a handful of naturally occurring, distinguishable probes are available, precluding efforts to visualize multicellular processes. Additionally, most bioluminescent photons are poorly tissue penetrant. To address these deficiencies, we have developed a set of unique luciferin analogs comprising pi-extended chromophores. These probes were predicted to emit more red-shifted light, enabling deep tissue imaging. Elongated pi-systems were achieved by introducing intervening aryl and vinyl motifs within the canonical luciferin framework. These scaffolds were also rotationally labile, offering a unique platform for achieving enzyme specificity. Light would be produced only when the luciferins were paired with luciferases capable of enforcing planarity. Native luciferases were found to be non-emissive with some of the analogs, setting the stage for orthogonal probe development. Mutants capable of processing the flexible luciferins were identified through Rosetta-guided enzyme design. The mutants were also found to be highly substrate specific, allowing them to be used in tandem with other engineered luciferase-luciferin pairs including one developed from our lab. The flexible luciferins and complementary enzymes are facilitating multicomponent and deep tissue imaging.