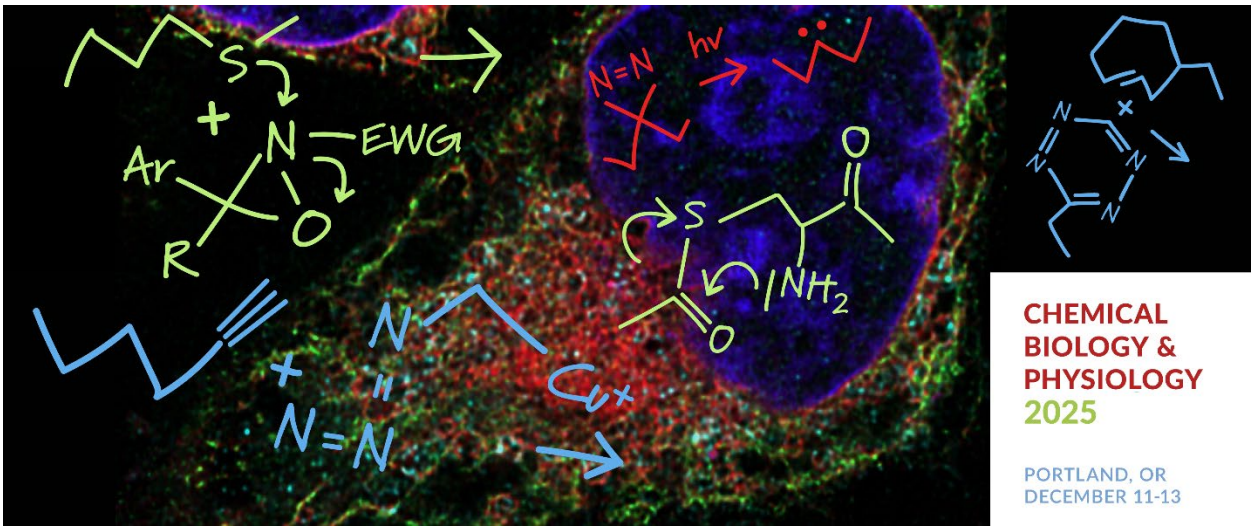


Program and Abstracts

December 11 – 13, 2025

Oregon Health & Science University

Portland, OR USA

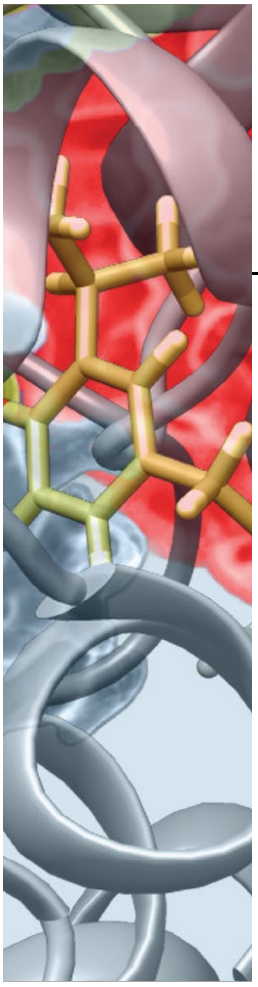


TABLE OF CONTENTS

KNIGHT
CANCER
Institute

School of
MEDICINE

Advion Interchim
scientific

ChemBioChem

SCIENCE STEERING COMMITTEE:

Kimberly Beatty, Ph.D., Oregon Health & Science University
Victoria DeRose, Ph.D., University of Oregon
James Frank, Ph.D., Oregon Health & Science University
Ryan Mehl, Ph.D., Oregon State University
Carsten Schultz, Ph.D., Oregon Health & Science University

SESSION CHAIRS:

Kimberly Beatty, Ph.D., Oregon Health & Science University
Victoria DeRose, Ph.D., University of Oregon
James Frank, Ph.D., Oregon Health & Science University
Leif Lindberg, Ph.D. Student, University of Oregon
Sarah McGee, Ph.D. Student, Oregon State University
Ryan Mehl, Ph.D., Oregon State University
Carsten Schultz, Ph.D., Oregon Health & Science University
Tatum Weishaar, Ph.D. Student, Oregon Health & Science University

EVENT PLANNERS:

Tonia Bishop
Amber Bruner
Lisa Gurung
Osvaldo Hernandez
Elie Inns
Amy Johnson
Claireese Kimmons
Sara Kopton
Jessica Lasneski
Denesa Lockwood
Shawna Rinne
Margaux Schwartz
Kris Thomason

SPECIAL THANKS TO:

Nick Crampton and Chase Thomas for AV support

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

THURSDAY – DECEMBER 11	
08:00 – 08:55	Check-in and Welcome
08:55 – 09:00	Opening Remarks

SESSION 1: CHEMICAL BIOLOGY TOOLS
Chair: Kimberly Beatty

09:05 – 09:35	**Xin Zhou**, Dana Farber Cancer Institute, Harvard Medical School *When antibodies become degraders*
09:35 – 10:10	**Ellen Sletten**, UC Los Angeles *In vivo imaging with shortwave infrared light*
10:10 – 10:25	**John Koberstein,** Janelia Research Campus *Molecular Tools and Imaging*
10:25 – 10:55	Break
10:55 – 11:30	**Ed Tate**, Imperial College, London *Targeting post-translational modification in drug discovery*
11:30 – 11:45	**Juner Zhang**, Princeton *Isotope-Enhanced Chemoproteomic Profiling of Endogenous Protein Serotonylation*
11:45 – 12:15	Flash Talks: 20 Poster presenters
12:15 – 13:15	Lunch Meet the speakers
13:15 – 14:45	Poster Session I - Sponsored by ChemBioChem

SESSION 2: CHEMICAL PHYSIOLOGY
Chair: James Frank

14:45 – 15:20	**Josh Levitz**, Weill-Cornell *Pinpointing therapeutic mechanisms of neuromodulatory GPCRs*
15:20 – 15:55	**Bianxiao Cui**, Stanford University *Membrane curvature-induced integrin adhesion and drug development*
15:55 – 16:10	**Tanya Hadjian**, University of California, Irvine *Continuous hypermutation and evolution of novel luciferase variants*
16:10 – 16:45	**David Olson**, UC Davis *Psychedelics and Related Plasticity-Promoting Neurotherapeutics*
16:45 – 17:00	Break

KEYNOTE SPEAKER
Sponsored by Amgen
Chair: Leif Lindberg

17:00 – 18:00	**Nancy Carrasco**, Vanderbilt University *The tale of the sodium/iodide symporter (NIS): From cloning to structure*

18:30 – 20:30	Reception

FRIDAY – DECEMBER 12

SESSION 3: CHEMICAL BIOLOGY OF LIPIDS
Chair: Carsten Schultz

09:00 – 09:35	**Summer Gibbs**, Oregon Health & Science University *Novel Fluorescent Contrast Agents to Improve Clinical Medicine*
09:35 – 10:10	**Jeremy Baskin**, Weill-Cornell *Revealing Mechanisms of Lipid Homeostasis Using Membrane Editing and Proximity Proteomics*
10:10 – 10:25	**Pankaj Gaur**, Université de Strasbourg, France *Environment-sensitive and red light activatable cage for biological application*
10:25 – 10:55	Break
10:55 – 11:30	**Benjamin Swarts**, Central Michigan State University *Synthetic lipids for probing the mycobacteria-host interface*
11:30 – 11:45	**Ben Barad**, Oregon Health State University *Surface Morphometrics 2.0: Incorporating Protein Localization and Density Sampling for Contextual Structural Analysis with Cryo-electron Tomography*
11:45 – 12:15	Flash Talks: 20 Poster presenters
12:15 – 13:15	Lunch - Meet the Speakers
13:15 – 14:45	Poster Session II - Sponsored by ChemBioChem

SESSION 4: CHEMICAL BIOANALYTICS
Chair: Vickie DeRose

14:45 – 15:20	**Gonzalo Cosa,** McGill University, Toronto *A window into lipid peroxyl radicals, peroxidation and lipid-derived electrophilic stress in cells*
15:20 – 15:55	**Yasuteru Urano**, Tokyo University *Development of novel chemistry-based and enzyme-driven theranostics technologies for cancer*
15:55 – 16:10	**Klara Gries**, Max Planck Institute for Medical Research, Heidelberg Next-generation self-labeling protein tags for multiplexed live-cell imaging and recordings
16:10 – 16:45	**Sheel Dodani**, University of Texas Dallas *An optical lense at chloride in biology*
16:45 – 17:00	Break

KEYNOTE SPEAKER
Sponsored by Leica Microsystems
Chair: Tatum Weishaar

17:00 – 18:00	**Chris Chang**, Princeton University *Engineering proteins to map and manipulate cells*

SATURDAY – DECEMBER 13

SESSION 5: CHEMICAL BIOLOGY OF PATHOGENS
Chair: Kimberly Beatty

09:00 – 09:35	**Stephan Sieber**, Technische Universitaet Muenchen *Chemical dysregulation to break bacterial resistance*
09:35 – 10:10	**Nicole Sampson**, University Rochester *Tuberculosis Target Discovery in Secondary Metabolic Pathways*

10:10 – 10:25	**Katelyn Alley:** University of Oregon *Dual-Protein and Click Expansion Microscopy Reveal Nanoscale Reorganization of the Nucleolus during Stress*
10:25 – 10:55	Break
10:55 – 11:30	**Fikadu Tafesse**, Oregon Health & Science University *Studying Host-Pathogen Interactions Using Lipid Probes*
11:30 – 11:45	**Peter Tonge**, Stony Brook University *Drug-target residence time and the post-antibiotic effect: Strategies to improve dosing regimens*
11:45 – 12:15	Flash Talks: 20 poster presenters
12:25 – 13:15	Lunch Meet the Editors
13:15 – 14:45	Poster Session III - Sponsored by ChemBioChem

SESSION 6: PROTEIN CHEMISTRY
Chair: Ryan Mehl

14:45 – 15:20	**Matt Francis**, UC Berkeley *Synthetically Modified Viral Capsids as Efficient Delivery Platforms for STING Agonists*
15:20 – 15:55	**Aiko Umeda,** Amgen *Genetic Code Expansion Enables Antibody–Protein Conjugates with Previously Inaccessible Architectures and Enhanced Functionality*
15:55 – 16:10	**Dora Kern,** HUN-REN Research Centre for Natural Sciences *Bioorthogonally activated protein labelling*
16:10 – 16:45	**George Burslem**, University of Pennsylvania *Intracellular protein editing*
16:45 – 17:00	Break

KEYNOTE SPEAKER
Sponsored by Advion Interchim Scientific
Chair: Sarah McGee

17:00 – 18:00	**Matt Bogyo**, Stanford University *Making it Stick: Using Covalent Ligands for Diverse Applications in Biology*

18:00	Poster Prize Presentations
18:10	Closing Remarks
18:15	Evening social

MODULAR STRATEGIES TO DECODE AND REWIRE CELL SURFACE SIGNALING

Xin Zhou

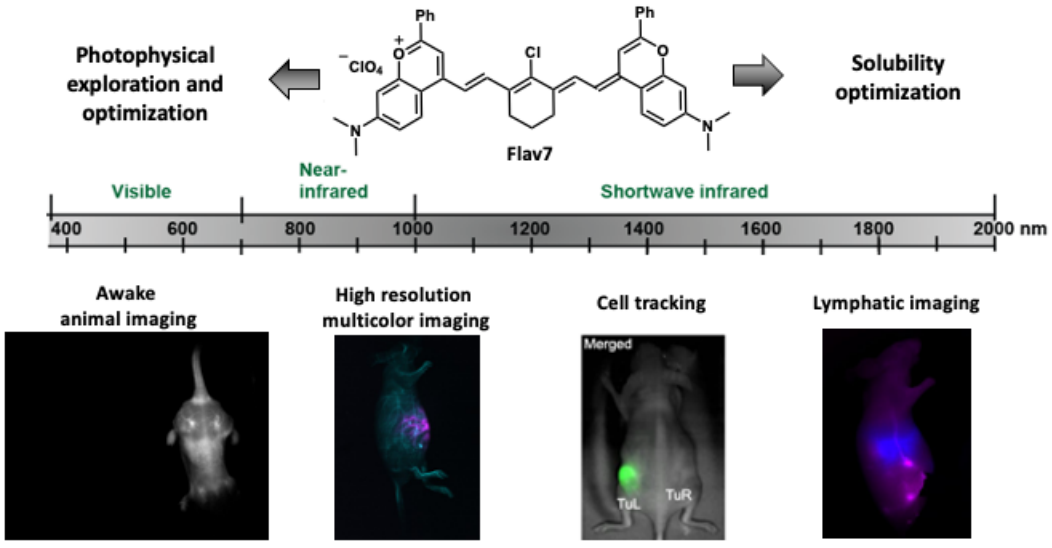
Cell surface proteins are key regulators of cell signaling, metabolism, and interactions with the surrounding environment. Promising strategies to modulate their activity include controlling endocytosis and regulating phosphorylation. While cells naturally use these processes to maintain membrane protein homeostasis and tune signaling outputs, harnessing them for therapeutic interventions is only beginning to be explored. In this talk, I will discuss how bispecific antibodies can be engineered to modularly control membrane protein internalization and signaling outcomes. I will also present a protein engineering platform for creating synthetic protein binders that recognize tyrosine-phosphorylated motifs in T cells. With these binders, we have developed sensors and biocircuits to map immune checkpoint signaling with spatiotemporal precision, uncover mechanisms of emerging inhibitors including anti–PD-1/VEGF bispecific antibodies, and reprogram signaling to build more effective cell therapies.

Non-invasively illuminating biology with shortwave infrared light

Ellen M. Sletten, University of California, Los Angeles

Fluorescence imaging is a central tool for visualizing complex biological systems, yet the contrast and resolution attainable *in vivo* is limited due to autofluorescence and light scattering at visible and near infrared (NIR) wavelengths. Recently, the shortwave infrared region of the electromagnetic spectrum (SWIR, 1000 – 2000 nm) has emerged as an optimal region for *in vivo* fluorescence imaging due to few endogenous SWIR chromophores and minimized scattering of light by tissue. While the SWIR demonstrates great promise, suitable materials are needed with emission at these low energies for the development of optical contrast agents. Namely, non-toxic organic small molecules with bright emission > 1000 nm are necessary to expand both the basic science and clinical applications of fluorescence imaging.

Our group has developed biocompatible polymethine fluorophores with shortwave infrared emission. We discovered a bright shortwave infrared fluorophore containing flavylium heterocycles that we deemed Flav7. We have systematically investigated Flav7 using physical organic chemistry approaches and can now predictably tune the absorption and emission properties. These insights have led to new SWIR fluorophores that enable multiplexed real time *in vivo* imaging and the fastest SWIR imaging to date.



Targeting Post-translational Protein Lipidation for Cancer Drug Discovery

Ed Tate, Imperial College London and The Francis Crick Institute

The Tate lab develops chemical biology probes to unlock drug discovery across post-translational modification (PTM) pathways and other hard-to-target mechanisms, with a strong focus on oncology. We combine chemical proteomics, high-throughput screening and chemical probe discovery to reveal PTM-driven processes that fuel tumour progression, immune evasion and therapy resistance.

Our goal is to open new druggable space in cancer by delivering actionable mechanisms, molecular tools and first-in-class therapeutic strategies. Recent highlights include the first cell-active activity-based probes for deubiquitinases, new platforms for detecting pathogenic secreted proteases, and the first comprehensive chemoproteomic maps of key PTM classes, offering mechanistic insights directly relevant to cancer.

Protein lipidation has become one of our most productive routes into cancer drug discovery. By dissecting N-myristoylation and S-acylation pathways using chemical biology, we have built discovery and target validation programmes centred on cancer, infectious disease and immunity. This work spans fundamental mechanistic biology through to clinical development, including the discovery of ultrapotent lipidation inhibitors with strong activity across diverse tumour models.

Our interests extend to next-generation therapeutic modalities such as targeted protein degradation and antibody-drug conjugates, where we are developing both mechanistic understanding and differentiated drug candidates. Translation of these platforms has been accelerated through spinouts including Myricx Bio (https://myricxbio.com/), pioneering ADC payloads based on our N-myristoyltransferase inhibitors, and Siftr Bio (https://siftr.bio/), applying our activity-based probe technologies to functional profiling of patient-derived cancer tissues. Together these companies have raised over $120 million to propel these discoveries towards patient impact.

Mechanism-guided identification of antidepressant G protein-coupled receptor drug targets

Josh Levitz, Weill Cornell Medicine, Biochemistry and Biophysics

The vast superfamily of G protein-coupled receptors (GPCRs) play critical roles in regulating neurophysiological and behavioral processes and, thus, serve as major drug targets for a wide range of neurological and psychiatric diseases. However, GPCR-targeting therapeutic approaches have typically been based on the serendipitous identification of symptom-alleviating compounds. Here we report a new platform for harnessing an understanding of the synaptic and circuit-level mechanisms underlying the pathophysiology and treatment of a psychiatric disorder to guide the development of new therapeutics. We first use a battery of chemical, genetic, and optical tools to decipher the mechanisms of action of the fast-acting antidepressant ketamine. We find that the behavioral effects of ketamine rely on agonism of mu-opioid receptors, which are enriched in somatostatin-expressing interneurons (Sst+ INs) in the medial prefrontal cortex (mPFC). Chronic stress drives presynaptic hypertrophy of mPFC Sst+ INs and excessive inhibition of pyramidal neurons, which is rescued by ketamine. Driven by these findings, we use transcriptomics to identify a plethora of mPFC Sst+ IN-enriched GPCRs and validate the antidepressant potential of novel GPCR targets. Synergistic targeting of multiple Sst+ IN-enriched GPCRs enables potent antidepressant-like responses with a reduced side effect profile compared to ketamine. Together this study provides new mechanistic insights with promise to improve antidepressant strategies while revealing a general approach to identifying new therapeutic GPCR targets for brain disorders.

Curvature-induced integrin adhesion and drug development

Bianxiao Cui, Stanford University

The extracellular matrix (ECM) is a fibrous scaffold composed of proteins like collagen, fibronectin, and elastin. When cells interact with these ECM fibers, their cylindrical geometry can induce local curvature in

the cell membrane. We discovered that such membrane curvature gives rise to a previously unrecognized class of integrin-mediated cell adhesion – **curved adhesion**. Curved adhesions bear lower mechanical forces than focal adhesions and exhibit distinct molecular compositions. Curved adhesions are especially prevalent in soft, 3D fibrous environments and form independently of the strong mechanical forces typically associated with focal adhesions. Interestingly, unlike focal adhesions, curved adhesions resist disruption by high affinity av integrin inhibitors. As a result, curved adhesions continue to support cancer cell invasion into 3D ECM in the presence of these inhibitors. These findings reveal a limitation of current integrin-targeting strategies and also highlight new opportunities for future therapeutic development.

Psychedelics and Related Plasticity-Promoting Neurotherapeutics

David E. Olson, University of California, Davis

Cortical atrophy underlies a wide variety of brain diseases including depression, post-traumatic stress disorder, and substance use disorder. Recently, our group discovered that psychedelics and related molecules, such as DMT, LSD, and MDMA, rapidly promote the growth of cortical neurons, providing a potential explanation for their long-lasting therapeutic effects after a single dose. However, these first-generation compounds suffer from one or more issues that limit their clinical scalability including hallucinogenic effects, cardiotoxicity, and psychostimulant properties. I will discuss the development of chemical and molecular tools for studying the mechanism(s) of action of psychedelics as well as our efforts to engineer non-hallucinogenic analogs of these compounds that produce similar sustained therapeutic behavioral effects after a single administration. Understanding the fundamental biochemical mechanisms that give rise to compound-induced neuroplasticity will be essential for developing safer and more effective neurotherapeutics for a variety of brain disorders.

“The tale of the sodium/iodide symporter (NIS): From cloning to structure”

Nancy Carrasco, Department of Molecular Physiology and Biophysics, Vanderbilt University

The sodium/iodide (Na+/I-) symporter (NIS) is the key plasma membrane protein that actively transports iodide into the thyroid gland, the first step in the biosynthesis of the thyroid hormones, of which iodine (oxidized iodide) is an essential constituent. NIS is also the molecule at the center of the highly successful treatment for thyroid cancer based on radioiodide, administered postthyroidectomy. I will discuss my extremely exciting journey investigating NIS with my group, from isolating the cDNA that encodes the protein and subsequently characterizing it at the molecular level to determining the 3-D structure of WT-NIS with diQerent substrates bound to it, and engineered NIS molecules with modified substrate specificities, by Cryo-EM—an undertaking that has opened up new and unexpected worlds of discovery.

Can Nerve-Specific Imaging Improve Clinical Medicine?

Summer Gibbs, Ph.D., Douglas Strain Endowed Professor, Biomedical Engineering, OHSU

Fluorescence guided surgery (FGS) is a nascent field, however with ~15,000 clinical FGS system distributed worldwide, its potential to specifically highlight tissues to be resected (e.g., cancer) and avoided (e.g., nerves) has been recognized and there are >125 ongoing clinical trials with novel contrast agents to leverage this clinical imaging technology. Iatrogenic nerve damage is arguably one of the most feared surgical complications as nerve injury is often permanent, leaving patients with pain, loss of function, disability and decreased quality of life. Near infrared (NIR) nerve-specific contrast agent(s) that are spectrally matched to the existing clinical FGS infrastructure have a direct path to clinical translation with broad surgical applicability. However, development of NIR nerve-specific probes has been a substantial challenge as these probes must be small enough to cross the tight blood nerve barrier, but have a

sufficient degree of conjugation to reach NIR wavelengths. Through a directed fluorophore medicinal chemistry approach, we have designed and developed first-in-kind, small molecule NIR nerve-specific fluorophores. Our team is currently working towards clinical translation of our novel probes as we explore the utility of nerve imaging for a variety of surgical indications including prostatectomy, neurosurgery, endocrine surgeries, head and neck surgeries and orthopedic indications.

Revealing Mechanisms of Phospholipid Homeostasis Using Membrane Editing Coupled to Proximity Labeling

Jeremy Baskin - Department of Chemistry and Chemical Biology and Weill Institute for Cell and

Molecular Biology, Cornell University

Lipid metabolism is subject to strong homeostatic regulation, but players involved in and mechanisms underlying these pathways remain mostly uncharacterized. I will describe a “Feeding–Fishing” approach coupling membrane editing using optogenetic lipid-modifying enzymes (feeding) with organelle membrane proteomics via proximity labeling (fishing) to elucidate molecular players and pathways involved in homeostasis of phosphatidic acid (PA), a multifunctional lipid central to glycerolipid metabolism. By performing proximity biotinylation using a membrane-tethered TurboID alongside membrane editing to selectively deliver PA to the same membrane, we identified numerous PA-metabolizing enzymes and lipid transfer proteins enriched in and depleted from PA-fed membranes. Subsequent analysis revealed local metabolism and interorganelle transport steps mediating PA homeostasis. More broadly, the interfacing of membrane editing with organelle membrane proteomics using proximity labeling represents a strategy for revealing mechanisms governing lipid homeostasis.

Synthetic lipids for probing the mycobacteria-host interface

Benjamin Swarts, Central Swarts

A window into lipid peroxyl radicals, peroxidation and lipid-derived electrophilic stress in cells

Gonzalo Cosa, Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, QC H3A 0B8, Canada

In this presentation I will describe fluorogenic (off to on) probes we have developed to monitor electron transport,1 lipid peroxidation,2 and electrophilic stress,3 in lipid membranes. I will portray live cell imaging work where we exploit newly developed activatable fluorogenic antioxidants2 and state-of-the-art imaging methodologies to monitor lipid peroxyl radicals under a series of pathological conditions. Secondly, I will touch upon the ability of cells to detoxify increasing lipid derived electrophile (LDE), exploring the link between lipid hydroperoxide accumulation, LDE formation and cell death.3 Here, I will describe a recently developed assay (ElectrophileQ3a,b) that enables live-cell assessment of the glutathione-mediated LDE conjugation and adduct export steps of the LDE detoxification pathway. The body of work provides molecular insight on the onset and progression of ferroptosis,2a a form of cell death where lipid peroxidation and ensuing electrophilic stress3a,b are exacerbated.

Refrences
1. a) J. Am. Chem. Soc. 2016, 138, 16388-16397. b) J. Am. Chem. Soc. 2016, 138, 11327-11334.
2. a) Nat. Chem., In press https://doi.org/10.1038/s41557-025-01966-x. b) Langmuir. 2023, 39, 1, 442-452. c) PLoS Biol. 2022, 20 (5). d) ACS Appl. Mater. Interfaces. 2022, 14, 11, 13872-13882. e) ACS Infect. Dis. 2020, 6, 2468-2477. f) J. Am. Chem. Soc. 2017, 139, 15801-15811. g) J. Am. Chem. Soc. 2012, 134, 10102-10113.
3. a) Proc. Nat. Acad. Sci. USA. 2024, 121, 21 e231761612. b) Chem. Sci. 2022, 13, 9727-9738. c) ACS Sensors. 2022, 7, 1, 166-174. d) J. Am. Chem. Soc. 2017, 139, 16273-16281.

Chasing Chloride in Cells

Sheel Dodani, Ph.D., Eugene McDermott, Department of Chemistry and Biochemistry, The University of Texas at Dallas

Fluorescent sensors have transformed how we visualize ion dynamics in living cells with spatial and temporal resolution. While much progress has been made in imaging biologically relevant cations, tools to monitor anions, particularly chloride, have lagged. Chloride is the most abundant biological anion and plays a central role in cellular physiology, functioning both as a counterion and a dynamic signaling ion. Motivated by its importance, researchers began developing fluorescent chloride sensors over three decades ago. Quinolinium-based dyes and green fluorescent protein (GFP)-derived sensors have stood the test of time, enabling high-throughput drug screening, discovery of new chloride-transporting proteins, and advanced neurobiological applications.

Despite these advances, chloride imaging remains in its infancy. In any given biological context, sensor properties can be both enabling and limiting. With this in mind, we are developing a modern arsenal of sensors designed to probe chloride in unique and previously inaccessible scenarios, with the overarching goal of democratizing chloride imaging.

In this presentation, I will highlight the design, mechanism, and cellular applications of fluorescent sensors that provide a direct-response readout of endogenous chloride levels.

Activity-Based Sensing: Using Chemical Reactivity to Decode Single-Atom Chemical Biology

Christopher Chang, **Princeton**

Traditional strategies for developing selective imaging reagents rely on molecular recognition and static lock-and-key binding to achieve high specificity. We are advancing an alternative approach to chemical probe design, termed activity-based sensing, in which we exploit inherent differences in chemical reactivity as a foundation for distinguishing between chemical analytes that are similar in shape and size within complex biological systems. This presentation will focus on activity-based sensing approaches to visualize dynamic fluxes of metal ions, reactive oxygen species, and reactive carbon species and their signal/stress contributions to living systems, along with activity-based proteomics probes to identify their biological targets at atomic scale. As a representative example of new biological lessons learned from these chemical probes, we are advancing a new paradigm of transition metal signaling, where metal nutrients like copper can serve as dynamic signals to regulate protein function by metalloallostery and promote copper-dependent cell growth and death pathways termed cuproplasia and cuproptosis, respectively. A second example of single-atom chemical biology comes from reversible redox interconversion between methionine and methionine sulfoxide, where activity-based probes reveal the landscape of site-specific proteome modifications at single amino acid resolution to identify new redox-dependent disease vulnerabilities.

Chemical Dysregulation to Break Bacterial Resistance

Professor Dr. Stephan A. Sieber, TUM, Lichtenbergstr. 4, 85747 Garching

Multiresistant bacterial pathogens such as Methicillin-resistant Staphylococcus aureus (MRSA) continue to drive severe, hard-to-treat infections and represent an escalating global health threat. Addressing this challenge requires the discovery of chemical entities that act through mechanisms distinct from those of existing antibiotics. In this presentation, I highlight our recent efforts to develop innovative antibacterial strategies aimed at disrupting core aspects of bacterial physiology. These approaches include the chemical disarming of pathogenicity, induction of intracellular stress through small-molecule modulators, AI-driven generation of structurally novel antibiotic candidates, and deliberate overactivation of endogenous bacterial enzymes to trigger autolytic pathways. To elucidate the underlying modes of action, we employed a suite of complementary techniques, with a particular emphasis on affinity-based protein profiling (AfBPP). Together, these studies demonstrate how leveraging unexplored chemical space, unconventional targets, and noncanonical mechanisms can provide fresh solutions for overcoming bacterial resistance and reinvigorating the antibiotic discovery pipeline.

Tuberculosis Target Discovery in Secondary Metabolic Pathways

Nicoles S. Sampson, University of Rochester

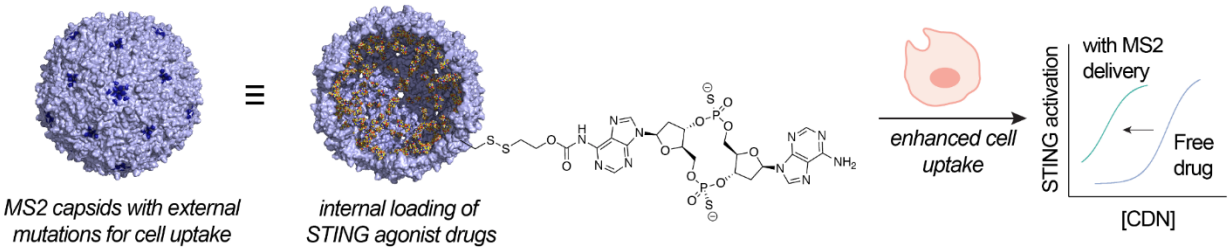
Cholesterol metabolism is one of the mechanisms by which mycobacteria, e.g., Mycobacterium tuberculosis (Mtb), survive and persist in their human host. An investigation of the entire cholesterol metabolism pathway in Mtb using a multi-pronged approach that includes elucidating enzyme function, establishing metabolite structure and activity has led to the identification of enzyme quaternary complexes with assemblies unique to mycobacteria that highlight mechanisms of control distinct from those in mammals or other families of bacteria.

STUDYING HOST-PATHOGEN INTERACTIONS USING LIPID PROBES

Lipids play central roles in infection and are critical at every stage of an intracellular pathogen's life cycle. Many viruses and other pathogens hijack host lipid pathways to enter cells, replicate, assemble, and evade immune responses. Yet, despite their importance, our understanding of how specific lipids contribute to these processes remains limited, largely because lipids are inherently difficult to visualize and biochemically track. In this seminar, I will present our approach to overcoming these challenges using chemically tailored lipid probes developed in collaboration with Carsten Schultz. These lipid analogs are minimally modified to retain their native biological function while carrying key features to incorporate a fluorophore for imaging purposes, and a biotin handle for affinity purification of interacting partners. Using proteomic and biochemical analyses, we employ these probes to map how pathogens rewire host lipid networks during infection and to uncover the molecular mechanisms that underlie lipid-dependent steps in pathogenesis. Eventually, these tools allow us to pinpoint vulnerable lipid-mediated interactions that can be exploited for antiviral or antibacterial drug discovery.

Synthetically Modified Viral Capsids as Efficient Delivery Platforms for STING Agonists

Matthew B. Francis, Department of Chemistry, University of California, and Lawrence Berkeley National Laboratory, Molecular Biophysics and Integrated Bioimaging Division, Berkeley, CA



Chemically-modified viral capsids are promising nanoscale platforms for the tissue-specific delivery of therapeutic cargo. Through appropriate surface modification, multiple copies of desired targeting groups can be displayed to direct these carriers to cell surface markers of interest. Additionally, the use of hollow structures allows drug molecules and radiolabels to be sequestered within the assemblies, protecting them from premature degradation and masking their influence on biodistribution. To realize this potential, we have developed a series of site-selective chemical reactions to convert the protein shells of a non-pathogenic virus, bacteriophage MS2, into a coordinated set of targeted delivery agents. In older work, we used these techniques to install peptides, antibody fragments, and DNA aptamers on their external surfaces, and we have modified their interiors to house MRI contrast agents, F-18 labels for PET imaging, therapeutic enzymes, and inorganic nanoparticles. In more recent work, we have used library screening methods to determine full fitness landscapes for these particles. In addition to providing information about the specific mutations that benefit or prevent protein self-assembly, these data have revealed locations

where new amino acids can be introduced to encourage cell binding. As a result, we now have capsids that show dramatically increased uptake into a variety of cancer cell lines. Coupling these mutations with interior modifications, we have recently developed these carriers into highly potent platforms for the delivery of STING pathway agonists for cancer immunotherapy. This presentation will review what we have learned about the chemical biology of MS2 viral particles, and will discuss their current and future potential as targeted agents for drug delivery.

GENETIC CODE EXPANSION ENABLES ANTIBODY–PROTEIN CONJUGATES WITH PREVIOUSLY INACCESSIBLE ARCHITECTURES AND ENHANCED FUNCTIONALITY

Aiko Umeda, Ph.D. – Amgen, USA

The ability to site-specifically control the design of antibody-protein conjugates (AbPCs) holds great potential for precision medicine by enabling the generation of multispecific modalities with synergistic properties greater than the sum of their parts. However, current approaches to generating AbPCs limit the chemical space and architectures available for their construction and thereby constraining their pharmacological properties. Thus, a strategy that enables precise control over the connectivity and architecture of each component -- antibody, protein, and linker -- would significantly enhance their clinical potential. Here, we employ genetic code expansion in combination with biorthogonal ligation to construct a series of structurally diverse AbPCs that deliver interleukin-2 to regulatory T cells in mice. We find that our topologically defined AbPCs exhibit a wide range of activities with a subset of the architectures exhibiting enhanced pharmacokinetic and pharmacodynamic properties compared to a linear antibody-IL2 fusion. Our results demonstrate that the largely untapped chemical space available to AbPCs holds immense potential for constructing conjugates with novel pharmacological properties and provides a viable approach for accessing this expanded chemical and design space.

INTRACELLULAR PROTEIN EDITING

George Burslem, University of Pennsylvania

The ability to study proteins in a cellular context is crucial to our understanding of biology. This presentation will describe a new technology for “intracellular protein editing”, drawing from intein-mediated protein splicing, genetic code expansion, and endogenous protein tagging. This protein editing approach enables us to rapidly and site specifically install new sequences, residues or chemical probes into a protein of interest. We demonstrate the power of this protein editing platform to edit cellular proteins, inserting epitope peptides, protein-specific sequences, and non-canonical amino acids (ncAAs). Importantly, we employ an endogenous tagging approach to apply our protein editing technology to endogenous proteins with minimal perturbation.

Making it Stick: Using Covalent Ligands for Diverse Applications in Biology

Matthew Bogyo, PhD. Stanford University School of Medicine, Department of Pathology, 300 Pasteur Dr. Stanford, CA 94305-5324.

Chemical tools that allow dynamic monitoring of enzyme activities through the formation of irreversible covalent bonds can be used for many diverse applications in basic biology as well as in drug discovery and clinical diagnostics. In this presentation, I will describe our efforts to design and build covalent binding small molecule probes using a range of techniques. Specifically, I will present recent advances in our development of fluorescent probes for applications in image guided cancer surgery as well as for the identification and imaging of several classes of serine hydrolases in pathogenic and commensal bacteria and cysteine proteases in viral pathogens. We believe many of these enzymes will represent valuable imaging and therapy targets that can be used to visualize and treat infections.

Accelerated fluorescent biosensor engineering using diverse libraries, high-throughput assays and machine learning

Dr. John Koberstein, Janelia Research Campus

Dr. Srinivas Turaga, Janelia Research Campus

Dr. Alison Tebo, Janelia Research Campus

Single fluorescent-protein biosensors are useful tools for probing biochemical activity at the level of single cells. The development and optimization of novel and existing biosensors is a laborious process. Typically, some combination of semi-rational design and directed evolution is used to produce an initial candidate sensor and then gradually improve its properties. While this methodology can result in useful tools, it is limited in the space of sequences explored and does not produce broad insights into the relationship between protein sequence and functional properties. To address these limitations, we sought to produce sequence-function data for a wide range of candidate biosensor designs. A highly diverse library was generated using an improved cloning method to produce all possible insertions of a fluorescent protein into a ligand binding domain with variable linker amino acids placed at the domain junctions using Golden Gate Assembly and pooled oligonucleotide synthesis. Sequences were assayed in parallel using a combination of FACS and DNA sequencing to measure the change in fluorescence intensity in response to ligand-binding. This approach was used to clone and assay thousands of candidate biosensor sequences which combine different ligand-binding domains and fluorescent proteins. This effort identified numerous high-performance biosensors with diverse and sometimes counterintuitive sequences. In addition to the discovery of functional sequences, measurements identify a large percentage of sequences that exhibit no fluorescence and many sequences that retain fluorescence but do not respond to the ligand. The resulting dataset can then be used to train machine learning models to predict functional insertion-sites and linker amino acids for combinations of ligand-binding and fluorescent protein domains. In a single round of screening, this high-throughput, high-diversity approach yields numerous high-function biosensors and a dataset that can be leveraged to guide future biosensor design efforts.

Isotope-Enhanced Chemoproteomic Profiling of Endogenous Protein Serotonylation

Dr. Juner Zhang, Princeton University

Ms. Kaylee Cappuccio, Princeton University

Mr. Zhenshu Tan, Peking University

Dr. Min Chen, Icahn School of Medicine at Mount Sinai

Mr. Joshua Sokol, Princeton University

Dr. Benjamin Weekley, Icahn School of Medicine at Mount Sinai

Dr. Jason Johansen-Leete, Princeton University

Prof. Ian Maze, Icahn School of Medicine at Mount Sinai and HHMI

Prof. Chu Wang, Peking University

Prof. Tom Muir, Princeton University

Serotonin is a ubiquitous and important signaling molecule in eukaryotic cells. Classical serotonin signaling pathways are often portrayed as receptor-dependent. In addition, it has become clearer that an alternative mechanism involving transamidation of cellular proteins plays important roles in many biological processes. In these processes, serotonin can be covalently attached to glutamine residues by cellular transglutaminases and exerts downstream functions. Over the past two decades, serotonylation has been found on a number of proteins such as RhoA, histone H3, and GAPDH. However, methods to globally profile the endogenous "serotonylome" remain lacking. Here, we report an isotope-enchanced chemoproteomic approach to profile endogenous serotonylation at site level. This approach takes advantage of: 1) a highly selective reactive warhead that differentiates serotonylated residuals over tyrosine and tryptophan residues – They are chemically similar to serotonin and significantly more abundant in the proteome. 2) An isotope-enhanced proteomic method that increases the fidelity and coverage in detecting this low-abundant modification. We envision our method can be a powerful tool to study serotonylation in neurodegenerative diseases and cancers,

wherein the exact roles of serotonin are often complex and overlooked.

Continuous hypermutation and evolution of novel luciferase variants

Ms. Tanya Hadjian, University of California, Irvine Dr. Ria A. Deshpande, University of California, Irvine Dr. Zachary R. Torrey, University of California, Irvine Ms. Amelia W. Hammond, University of California, Irvine Dr. Rosana S. Molina, University of California, Irvine Dr. Kevin K. Ng, University of California, Irvine Prof. Chang C. Liu, University of California, Irvine Prof. Jennifer A. Prescher, University of California, Irvine

Bioluminescence is a useful imaging modality for many applications, such as biosensing. Several engineered luciferases have been developed, and the collection continues to grow as new applications are pursued. Many of these variants were discovered through directed evolution and manual screening. This current workflow for luciferase optimization, while successful, remains laborious and inefficient. Light emission from bioluminescence is relatively weak, precluding standard selections or rapid sorts with common instruments (e.g., flow cytometry). Mutant libraries are generated in vitro and screened, "winning" mutants are picked by hand, and the isolated sequences are subjected to additional rounds of mutagenesis and screening. Here, we present a streamlined platform for luciferase engineering that removes the need for manual library generation during each cycle. We purposed an orthogonal DNA replication (OrthoRep) system for continuous hypermutation of a well-known luciferase (GeNL). Short cycles of culturing and screening were sufficient to evolve the enzyme, with no repetitive manual library generation necessary. New GeNL variants were identified that exhibit improved light outputs with a non-cognate and inexpensive luciferin. We further characterized the novel luciferases in cell models. Collectively this work establishes OrthoRep and continuous hypermutation as a viable method to engineer luciferases, and sets the stage for more rapid development of bioluminescent reporters.

Environment-sensitive and red light activatable cage for biological application

Dr. Pankaj Gaur, Université de Strasbourg, France

Ms. Chiara Gigliucci, Université de Strasbourg, France

Ms. Julie Obeid, Université de Strasbourg, France

Dr. Andrey Klymchenko, Université de Strasbourg, France

Photocaging/caging is a technique in which a molecule of interest can be rendered biologically inert by covalent attachment with a cage. Illumination of the caged compound results in a rapid and repetitive concentration jump of biologically active molecule leading to photo-controlled biological effect, such as activation of cellular receptors.1, Therefore, photomanipulation of cellular chemistry using caged compounds has emerged as a powerful technique to provide a highly precise and spatiotemporal control over the release of various biomolecules, second messengers and drugs. Despite the great achievements of the cage compounds in the biological manipulations, the need of UV or violet light for their stimulations makes them incompatible with the applications requiring deep tissue penetration. Moreover, these cages are exclusively controlled by light, and hence suffer from the major issue of off-target biological effects. It necessitates further development to explore new chemical strategies to design cages capable of cell-specific and microenvironment-controlled photoactivation. Variation in the cellular microenvironment, namely polarity and viscosity, is the hallmark of several pathological conditions such as cancer and neurodegeneration. By exploiting these differences, new cages could recognize target cells and tissues, and thereby helping to minimize the off-target effects. In this regard, we designed and synthesized a series of meso-methyl BODIPY-based cages. By exploiting the environmentally-regulated excites states of the solvatochromic probes (Fig.1), we structurally engineered, for the first time, an environment-sensitive BODIPY cage through systematic tuning of its push-pull architecture. A comprehensive structural-activity relationship study including photophysical and photochemical investigations as well as kinetic and mass analyses were performed to demonstrate the influence of electron-donating substituents on the environment-sensitive photochemical performance of BODIPY cages. Among the synthesized compounds, one exhibited the microenvironment-enabled light up (fluorogenicity) accompanied by photorelease in apolar media. These results indicate that

improving push-pull properties of BODIPY cage enables environment-sensitive uncaging through the generation of a high energy excited state in apolar media (Fig.1). Taken together, environment-sensitive cage is expected to offer a modular design strategy for new photochemical tools for imaging and therapeutic applications in the field of photopharmacology.

Surface Morphometrics 2.0: Incorporating Protein Localization and Density Sampling for Contextual Structural Analysis with Cryo-electron Tomography

Dr. Benjamin A Barad, Oregon Health & Science University

Ms. Michaela Medina, The Scripps Research Institute

Ms, Ya-Ting Chang, The Scripps Research Institute,

Dr Mark Frank, Oregon Health & Science University

Mr. Zidan Khan, University of Tennessee, Knoxville

Dr. Frederick A Heberle, University of Tennessee, Knoxville

Dr. M Neal Waxham, MD Anderson Cancer Center

Dr. Danielle A Grotjahn, The Scripps Research Institute

Cellular cryo-electron tomography captures detailed three-dimensional snapshots of the interior of cells in a fully native cryopreserved state, revealing details of both protein localization and structure as well as the ultrastructural arrangement of the organellar membranes and cytoskeletal filaments within the cell. A major challenge to achieving the potential of the technique is connecting the protein and organellar scales to enable true contextual structure analysis. The Surface Morphometrics toolkit was initially developed as a tool to build quantifiable models of membranes within cells, enabling routing ultrastructural geometry analysis. We have developed new extensions to this toolkit to facilitate routine contextual structure/ultrastructure analysis, which we are releasing as Surface Morphometrics 2.0. These advances include membrane-guided density sampling and routine measurement of membrane thickness within cells, as well as a routine for robustly identifying changes to membrane ultrastructure that are associated with membrane proteins. These new tools are available in a fully pipelined command line interface as well as in a newly developed graphical user interface based on napari.

Toward multiplexed recordings with split self-labeling protein tags

Klara Gries, Department of Chemical Biology, Max Planck Institute for Medical Research, Heidelberg, Germany

Dr. Yin-Hsi Lin, Department of Chemical Biology, Max Planck Institute for Medical Research, Heidelberg, Germany

Dr. Julien Hiblot, Department of Chemical Biology, Max Planck Institute for Medical Research, Heidelberg, Germany

Prof. Kai Johnsson, Department of Chemical Biology, Max Planck Institute for Medical Research, Heidelberg, Germany & Institute of Chemical Sciences and Engineering (ISIC), École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Molecular recorders capture transient cellular events such as neuronal activity or calcium dynamics by transforming them into permanent marks for post-hoc analysis, thereby enabling the investigation of large cell populations with single-cell resolution. Our recently developed chemogenetic recording strategy based on an engineered split-HaloTag facilitates the modular construction of recorders for different biochemical activities by exchanging the sensing domain. With a set of split-HaloTag-based recorders available for calcium1, protein-protein-interactions1, GPCR signaling1 and kinase activity2, however, the current toolbox lacks the possibility to combine multiple recorders in one measurement for multiplexed recordings.

We aim to expand the split-HaloTag-based recorder strategy by introducing split variants of the orthogonal SNAP- and CLIP-tag, so that the recorder identity can ultimately be distinguished based on the substrate choice. Since previous split versions of SNAP-tag do not meet key criteria for this recorder strategy such as fast labeling upon reversible complementation and tunable affinity of the two split parts, we used the recently optimized SNAP-tag23 and CLIP-tag2 as a starting point for engineering. Both SNAP-tag2 and CLIP-tag2 exhibit labeling kinetics similar to HaloTag7, while remaining orthogonal. The superior performance of SNAP-tag2 and CLIP-tag2 compared to their ancestors

has been demonstrated in multicolor confocal and super-resolution stimulated emission-depletion (STED) microscopy in mammalian cells and yeast.3 Additionally, different protein variants are available for multiplexed fluorescence lifetime imaging (FLIM).

We evaluated different split designs in vitro and via yeast surface display. The identified lead candidates show complementation-dependent labeling in vitro, on the yeast surface and in live cells. After further optimization using rational design and directed evolution, these orthogonal split self-labeling proteins are expected to enable multiplexed recordings of different cellular activities alongside the split-HaloTag-based recorders – thus, enhancing our ability to dissect complex signaling cascades and their regulators

References
1 M. C. Huppertz, J. Wilhelm, V. Grenier, et al. Science (2024).
2 D. Sun, S. W. Ng, Y. Zheng, et al. Nat Chem Biol (2025).
3 S. Kühn, V. Nasufovic, J. Wilhelm, et al. Nat Chem Biol (2025).

Dual-Protein and Click Expansion Microscopy Reveal Nanoscale Reorganization of the Nucleolus during Stress

Ms. Katelyn R. Alley

Ms. Katelyn M. Wyatt

Mr. Adam C. Fries

Prof. Victoria J. DeRose

Platinum based anticancer agents such as oxaliplatin are cornerstone chemotherapeutics that exert cytotoxicity through the formation of Pt(II) adducts with cellular biomolecules. FDA approved cisplatin induces the DNA damage response pathway while oxaliplatin uniquely induces the nucleolar stress pathway. The nucleolus, a membraneless organelle, is central to ribosome biogenesis and stress sensing, yet the nanoscale changes in the cells during nucleolar stress remain poorly resolved. We applied dual protein 4x Expansion Microscopy (dual-proExM) in combination with click Expansion Microscopy (click-ExM) to capture images at the highest resolution reported for the nucleolus of ~45 ± 2 nm, enabling nucleolar reorganization on treatment with small molecules. ExM enabled quantitative mapping of nucleolar proteins RPA194, fibrillarin, and NPM1 during progressive stress stages. We observed condensation of fibrillar center components, relocation to nucleolar caps and redistribution of NPM1 to the nucleoplasm. Prolonged stress produced discrete fibrillarin and NPM1 positive nuclear foci, indicating severe nucleolar disruption. Combining ExM with 5-ethynyl uridine labeling of nascent RNA, we found rRNA synthesis declines early and ceases as nucleolar disassembly progresses. This study defines the nanoscale sequence of nucleolar reorganization and links nucleolar architecture to transcriptional activity, establishing ExM as a powerful tool for studying the membraneless nucleolus. Our ongoing work aims to investigate how nucleolar disruption affects cellular survival pathways, the reversible formation of the nucleolus during transient stress, and the integration of machine learning approaches to monitor dynamic changes in nucleolar structure.

Drug-target residence time and the post-antibiotic effect: Strategies to improve dosing regimens

Prof. Peter Tonge, Stony Brook University

Ms. Siobhan Cohen, Stony Brook University

Prof. Deborah Hung, Broad Institute of Harvard and MIT

Dr. Samantha Miranda, Broad Institute of Harvard and MIT

The post-antibiotic effect (PAE) is the persistent suppression of microbial growth following the removal of antimicrobial therapy. In general, antibiotics that generate a PAE are dosed less frequently, and thus, strategies to increase the PAE may improve drug safety. The PAE can arise if the drug is still bound to the target following eliminatiuon, and we have shown that the magnitude of the correlation between drug-target residence time and PAE provides insight into target vulnerability, which is the amount of target that the drug must engage to cause the desired pharmacological effect. In addition, we have also shown that rapid target resynthesis limits the extent of the PAE. It follows that strategies to increase target vulnerability and/or reduce target synthesis rates should lead to the development of better, safer therapies. Several systems will be presented which focus on compounds that are time-dependent inhibitors

with long residence times on their target. This includes the benzoxaborole inhibitors of leucyl-tRNA synthetase (LeuRS), and inhibitors of UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase (LpxC). The benzoxaborole drugs epetraborole and ganfeborole are in clinical trials for the treatment of lung diseases caused by Mycobacterium tuberculosis and M. avium complex (MAC). We have studied the residence time and PAE of these compounds and have determined that decreasing the rate of LeuRS synthesis leads to an increase in PAE. We have also shown that the selectivity of ganfeborole for M. tuberculosis LeuRS (mtLeuRS) compared to E. coli LeuRS (ecLeuRS) is due to the high vulenerability of mtLeuRS. We are now using gene knockdown and knockout strategies to identify novel targets that modulate the PAE generated by inhibitors of essential enzymes in E. coli and Pseuodomonas. This includes the E. coli Keio collection of ~4000 deletion strains and CRISPRi of essential genes in Pseuodomonas. We are measuring the rate of synthesis using pSILAC and determining mechanistic approaches to increase target vulnerability.

Bioorthogonally activated protein labelling

Ms. Dóra Kern, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary

Dr. Attila Kormos, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary

Dr. Márton Bojtár, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary

Dr. Tímea Imre, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary

Dr. Krisztina Németh, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary

Dr. Péter Kele, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary

Despite the rapid evolution of antitumor tools, problems such as systemic cytotoxicity, non-specific binding of covalent inhibitors or short residence time of non-covalent inhibitors need to be addressed. In line with our ongoing research interest, we aimed at challenging the limitations of bioorthogonal fluorogenic design strategies, by redefining the role of the bioorthogonal tetrazine moiety. More particularly, we applied a click-to-release tetrazine (crTz) to get access to the bioorthogonally controlled release of a pro-fluorescent electrophilic quinone methide species that was expected to be captured by a proximal nucleophile of the target protein. While conducting these experiments using a trans-cyclooctene (TCO) bearing reversible enzyme (carbonic anhydrase, CA) inhibitor, we serendipitously discovered an intriguing parallel reaction. A more detailed analysis of the reaction products suggested the covalent modification of the protein with a pyridazine moiety, which brought our attention to a so far neglected species of click-to-release reaction schemes. We concluded that the reaction of the crTz reagent with the TCO-inhibitor conjugate triggers an electron cascade, leading to the generation of a highly electrophilic pyridazine methide species. This moiety is subsequently captured by a nucleophilic amino acid side chain of the aforementioned enzyme. Importantly, this capture results in the covalent anchoring of the so far non-covalent inhibitor to the enzyme, rendering the reversible inhibitor irreversible.

The possibility of turning a reversible inhibitor to a covalent inhibitor upon chemical control could address the above mentioned challenges in inhibitor design. LC-MS, MS-MS and competition measurements with acetazolamide confirmed covalent ligation of the non-covalent inhibitor to the protein. Specific, ligand directed labelling was also demonstrated in the presence of trypsin inhibitor or β-Lactoglobulin, without CA. To expand the scope of the applicable inhibitors, ligand binding will also be tested by other tumor-associated enzymes such as bromodomain-containing protein 2 (BRD2).

References
[1] D. Kern et al. Eur. J. Org. Chem. 2024, 27, e202400541.
[2] A. H. A. M. van Onzen et al. J. Am. Chem. Soc. 2020, 142, 10955–10963.

2 - A DIA-based chemoproteomic platform for mapping lysine reactivity across the human kinome

Daniel S. Brush, University of Washington
Dr. Dustin J. Maly, University of Washington

Protein lysine residues play diverse roles in catalysis, regulation, and ligand binding, yet their intrinsic chemical reactivity remains poorly characterized. Existing lysine-reactivity methods rely on stochastic data-dependent acquisition (DDA) and detect only adducted peptides, resulting in incomplete and biased coverage. These limitations are especially pronounced for protein kinases, which are present at low cellular abundance and therefore underrepresented in global proteomic datasets. Consequently, lysine reactivity across the human kinome remains largely unexplored. To address this gap, we developed KIB-DIA-LRP, a data-independent acquisition (DIA) chemoproteomic workflow that integrates kinase inhibitor bead (KIB) enrichment with a dual-peptide readout of lysine modification. The first readout quantifies covalently modified peptide intensities that increase with labeling concentration, while the second monitors corresponding decreases in native lysine-containing tryptic peptides. Both are normalized to lysine-free peptides that control for LFQ variability and report kinase enrichment levels. Consistent kinase quantification across all labeling conditions indicates that proteins remain bead-bound and properly folded during electrophile treatment, preserving biologically relevant reactivity profiles. This approach expands kinome coverage 1.5-fold over DDA workflows, routinely detecting over 100 kinases and quantifying over 1,000 lysine sites—approximately fivefold deeper lysine coverage per kinase compared to existing proteome-wide methods. Using this platform, we systematically profiled lysine reactivity with distinct electrophiles to dissect how local environment and reaction mechanism shape labeling outcomes. Formaldehyde dimethylation established a baseline of accessibility-driven reactivity, favoring lysines in loop regions over α-helices or β-strands. In contrast, pentafluorophenyl acetate (PFPA) exhibited striking orthogonal selectivity, preferring α-helical lysines and strongly correlating with known post-translational modification sites—revealing that electrophile mechanism, rather than solvent exposure alone, dictates reactivity. Finally, fragment-like aminophiles produced unique, pocket-selective modification signatures with minimal overlap. Among these, a substituted squarate (ASQ) displayed a "Goldilocks" profile: moderate global reactivity but pronounced enrichment for high-magnitude responders that map to structured binding pockets in kinases such as CHUK and ABL2. Together, these results establish KIB-DIA-LRP as a robust platform for quantitative, kinome-wide lysine reactivity profiling. By resolving electrophile-dependent selectivity and identifying lysine-directed scout fragments, this approach provides a foundation for druggable pocket discovery and expands covalent inhibitor design beyond cysteine-centered paradigms.

3 - Deep Learning Driven Image Analysis for Tracking Nucleolar Morphology

Mr. Leif E. Lindberg‡, University of Oregon
Ms. Katelyn R. Alley‡, University of Oregon
Ms. Sylvia H. Kennerly, University of Oregon
Mr. Ian Reynolds, University of Oregon
Prof. Victoria J. DeRose*, University of Oregon

The nucleolus, a membrane-free organelle responsible for ribosome production, is highly sensitive to stress and shows distinct structural changes when cells are treated with chemotherapeutic drugs. Some drugs like the FDA approved oxaliplatin cause permanent nucleolar stress, likely through covalent binding to biomolecules, while others such as actinomycin D induce reversible stress through non-covalent interactions. The mechanisms that lead to nucleolar stress by small molecules and mechanisms that allow the nucleolus to recover from reversible stress remain unclear. A bottleneck in such investigations is that identifying stress traditionally requires slow manual imaging and immunostaining. To address this, we developed deep learning models that automatically detect and quantify nucleolar stress directly from microscopy images. These models perform comparably to standard biochemical assays and can classify cells as stressed or unstressed using only a single RNA-selective dye. Inclusion of additional protein markers capture finer differences across cell populations. We are now using this platform to

study how cells recover from reversible nucleolar stress, providing a high-throughput approach to explore nucleolar function, resilience and responses to chemotherapy.

#4 - Rewiring Cell-Cell Synapses with Engineered Scaffold Proteins

Dr. Nick Y. Shin, University of California, San Francisco
Dr. Kiersten M. Ruff, Washington University in St. Louis
Prof. Rohit V. Pappu, Washington University in St. Louis
Prof. Wendell A. Lim, University of California, San Francisco

Formation of specialized cell-cell interfaces is essential for diverse biological processes, from neuronal synapses to immune recognition. These interfaces contain condensate-like assemblies of scaffold proteins that organize signaling molecules and cytoskeletal components, yet the underlying organizational mechanisms remain poorly understood.

Here we demonstrate that a minimal synthetic scaffold protein drives partner cell-specific synapse formation. When co-expressed with a weakly-interacting receptor, this intracellular scaffold selectively assembles into punctate structures exclusively at contact sites with partner cells expressing cognate binding partners. Through integrated experimental and computational approaches, we reveal that synthetic scaffolds form dynamic membrane-localized condensates driven by weak multivalent interactions and specific domain architectures, functioning as signaling hubs.

We demonstrate functional control by fusing scaffold proteins to actin and microtubule regulators, achieving targeted enrichment of filamentous actin and microtubule bundles at specific cell-cell interfaces. These synthetic interfaces also induce specialized cell-cell communication, including contact-mediated polarity establishment.

Our findings establish core design principles for organizing cell-cell synaptic complexes and provide a modular platform for programming cellular interactions with potential applications in cell-based therapeutics.

(Graphic abstract on the next page)

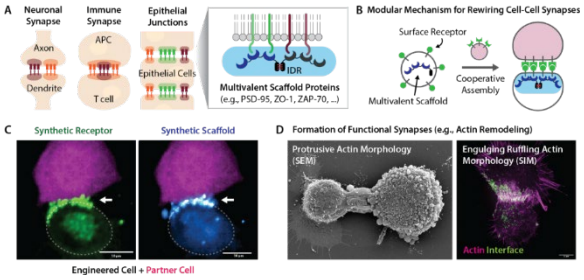


Figure 1. Scaffold protein-mediated cooperative assembly is the modular mechanism for creating natural cell-cell junctions and rewiring synthetic cell-cell synapses. (A) Natural cell-cell interfaces, such as neuron-neuron wiring and immunological synapses, create specific supramolecular signaling clusters mediated by multivalent scaffold proteins. (B) We postulated that scaffold protein-mediated cooperative assembly might be a modular mechanism underlying cell-cell communication and can be co-opted for synthetic biology. (C) When co-expressed in mouse fibroblasts, the cognate pair of synthetic receptor and scaffold protein creates co-localized condensates only at a specific contact point with a partner cell. (D) These multivalent scaffold proteins can indeed form functional cell-cell synapses by recruiting specific effector molecules and remodeling cytoskeletons.

#5 - Rescue of KATP channel nonsense mutations using anticodon-edited transfer RNAs

Dr. Yi-Ying Kuo, Oregon Health and Science University, Portland_
Ms. Zhongying Yang, Oregon Health and Science University, Portland
Dr. Joseph J. Porter, University of Rochester School of Medicine and Dentistry, Rochester
Prof. John D. Lueck, University of Rochester School of Medicine and Dentistry, Rochester
Prof. Show-Ling Shyng, Oregon Health and Science University, Portland

Background: Pancreatic β-cell KATP channels composed of sulfonylurea receptor 1 (SUR1) and Kir6.2 subunits encoded by ABCC8 and KCNJ11, respectively, play a critical role in regulating insulin secretion. More than 100 premature stop codon (PTC, a.k.a. nonsense) mutations have been identified in ABCC8 and KCNJ11, which result in loss of β-cell KATP channel function and cause congenital hyperinsulinism. Diazoxide, a KATP channel activator, and the only FDA

approved pharmacological treatment for congenital hyperinsulinism, is ineffective for patients with PTC mutations due to a lack of expression of functional KATP channels. Anticodon-edited transfer RNAs (ACE-tRNAs) suppress PTCs, resulting in translation of full-length protein. ACE-tRNAs have emerged in recent years as a potential therapy for diseases caused by nonsense mutations. For example, ACE-tRNAs have been shown to rescue the expression and function of cystic fibrosis transmembrane conductance regulator (CFTR) PTC mutations associated with cystic fibrosis. The applicability of ACE-tRNAs to rescue the expression and function of congenital hyperinsulinism-causing KATP channel PTC mutations has not been determined. Aims: Our study aims to evaluate the efficacy of ACE-tRNAs to rescue expression and function of KATP channels containing PTC mutations known to cause congenital hyperinsulinism. Methods: SUR1 or Kir6.2 cDNAs containing PTC mutations were transiently co-transfected with wild-type Kir6.2 or SUR1 cDNAs, respectively, along with ACE-tRNAs in COSm6 cells. 48 hours after transfection, protein expression was assessed by western blot, and channel function was evaluated using Rb^+ efflux assay and inside-out patch-clamp electrophysiology. Results: Five SUR1 PTC mutations: R248X, R705X, R934X, C1150X and R1539X were selected for initial experiments to test the feasibility of rescue by ACE-tRNAs, including ACE-tRNAArg and ACE-tRNACys, which incorporate the endogenous amino acid (Arg or Cys) at the PTC site, as well as ACE-tRNALeu, which has been shown to have the broadest PTC suppression applicability. Western blots demonstrate that while a negative control composed of a scrambled ACE-tRNA sequence failed to rescue full-length SUR1 PTC mutant protein expression, ACE-tRNAs that incorporate native amino acids or Leu at the PTC sites led to expression of full-length SUR1. Functional evaluations using the Rb+ efflux assay and electrophysiology confirmed significant functional rescue of channel activity in cells co-transfected with PTC-containing SUR1, wild-type Kir6.2, and ACE-tRNAs incorporating native amino acids. ACE-tRNALeu, which incorporates a non-native Leu at these sites, also significantly increased the function of mutant channels, with the exception of R1539X, in Rb+ efflux assays and electrophysiology experiments. Upon establishing the efficacy of ACE-tRNAs to rescue full-length channel expression and function, additional SUR1 PTC mutations (a total of 92 including the five tested in the initial experiments) and Kir6.2 PTC mutations (a total of 7) were screened for their response to an optimized ACE-tRNALeu, which has superior efficiency in suppressing PTCs. Rb+ efflux assay results showed that a majority of the SUR1 PTC mutations (60) and four of the seven Kir6.2 PTC mutations were functionally rescued by optimized ACE-tRNALeu. Conclusion: Our results demonstrate that ACE-tRNAs are effective in rescuing the expression and function of SUR1 and Kir6.2 PTC mutations. Delivery of ACE-tRNAs may serve as a potential molecular therapy for congenital hyperinsulinism caused by KATP PTC mutations. While ACE-tRNALeu is effective at rescuing full-length expression and function of a majority of SUR1 and Kir6.2 PTC mutations, conversion of some PTC sites to a non-native leucine amino acid may compromise KATP channel function. This highlights that the use of ACE-tRNAs that incorporate non-native amino acids at PTC sites needs to be evaluated on a case-by-case basis.

#6 - To label, detect and target newly synthesized proteins for synergistic anticancer activity

Dr. Jian Wang, Oregon Health & Science University
Dr. Bo Chao, Oregon Health & Science University
Mr. Jake Piesner, Oregon Health & Science University
Dr. Felice Kelly, Oregon Health & Science University
Dr. Stefanie Kaech Petrie, Oregon Health & Science University
Dr. Xiangshu Xiao, Oregon Health & Science University
Dr. Bingbing X. Li, Oregon Health & Science University

Protein synthesis and subsequent delivery to the target locations in cells are essential for their proper functions. Newly synthesized proteins and existing proteins have differential properties that can potentially be exploited for targeting to maximize therapeutic potentials. Methods to label and distinguish newly synthesized proteins from existing ones are critical to assess their differential properties, but such methods are lacking. We describe the first chemical genetics-based approach for selective labeling of existing and newly synthesized proteins that we termed as CG-SLENP. Using HaloTag in-frame fusion with lamin A (LA), we demonstrate that the two

pools of proteins can be selectively labeled using CG-SLENP in living cells (JACS Au, 2024, https://doi.org/10.1021/jacsau.4c00461). We further employ our recently developed selective small molecule ligand, LBL1 for LA to probe the potential differences between newly synthesized and existing LA. Our results show that LBL1 can differentially modulate these two pools of LA. The CG-SLENP approach described here provides a novel platform to selectively investigate the properties of newly synthesized proteins and the existing ones. It provides a snapshot of cellular activity, capturing dynamic changes in protein production—especially in response to drug treatment.

#7 Designing Ubiquitin Tools from Host/Pathogen Interactions

Jasleen Kaur Sidhu, Tyler Franklin, Jonathan Pruneda

Ubiquitin is a eukaryotic post-translational modifier that controls diverse cellular outcomes including proteasomal degradation, autophagy, and DNA damage response. The diversity in signalling outcomes is driven by an assortment of polymeric ubiquitin chains attached via lysine residues, whereby only some of the topologies are well studied. Interestingly, bacteria lack an endogenous ubiquitin system, but they have evolved secreted effectors that hijack the host ubiquitin system during infection and regulate the formation of various topologies of ubiquitin chains. Through studying how bacteria manipulate ubiquitin signals to suppress immune responses, we can gain more information on the different chain topologies and their effect on cellular pathways. One such effector secreted by Enterohemorrhagic Escherichia coli (EHEC), known as NleL, is a bacterial ligase that has been shown to target host caspase 4/5 and ROCK 1/2 for proteasomal degradation through attachment of polyubiquitin chains. Caspase 4/5 constitute the noncanonical inflammasome that activates pyroptosis while ROCK 1/2 regulate actin dynamics required for downstream intestinal epithelium cell extrusion. NleL builds canonical proteasomal degradative signals (K48 ubiquitin), but K6 ubiquitin, an 'atypical' signal, is equally prevalent. The 'atypical' K6 signal has been understudied relative to canonical ubiquitin signals as there has been no human enzymes that regulate K6 chains to the extent that NleL does. Through CryoEM structural analysis, we can identify the molecular details of the NleL complex with ubiquitin and its substrates caspase 4 or ROCK 2, pinpointing unique features that generates specificity for K6 polyubiquitin chains. Through structure-guided mutagenesis, we generated mutants of NleL that allow us to toggle its specificity between K48 and K6 polyubiquitin chains. Using these mutants, I investigated whether the NleL substrates caspase 4 and ROCK2 can be selectively targeted by K6 or K48 chains. Moving forward, these NleL mutants will be optimized to probe the influence of K6 signalling in immune response suppression during EHEC infection.

#8 - Reader-Writer E3 Ligases RNF114 and RNF166 Couple MARUbylation to K11-Linked Polyubiquitylation

Dr. Kapil Upadhyaya, Oregon Health & Science University
Dr. Rachel E Lacoursiere, Oregon Health & Science University
Ms. Jasleen Kaur Sidhu, Oregon Health & Science University
Dr. Ivan Rodriguez Siordia, Oregon Health & Science University
Dr. Daniel S Bejan, Oregon Health & Science University
Prof. Michael S Cohen, Oregon Health & Science University
Prof. Jonathan N Pruneda, Oregon Health & Science University

Post-translational modifications (PTMs) such as ADP-ribosylation and ubiquitylation frequently intersect to regulate protein fate and signaling. We recently identified mono-ADP-ribosylation-dependent ubiquitylation (MARUbylation) as a novel crosstalk mechanism in which a mono-ADP-ribose (ADPr) moiety serves as a non-canonical site for ubiquitin attachment (Bejan et al., *EMBO J.*, 2025). However, the enzymatic machinery that installs and propagates these hybrid ubiquitin-ADPr conjugates remained unknown.

Here, we show that the Deltex E3 ligase DTX2 and the RING-type ligase RNF114 cooperate to generate and extend MARUbylation on PARP7 in cells. DTX2 catalyzes the formation of a mono-ubiquitin-ADPr ester (MARUbe) in a PARP7 activity-dependent manner, while RNF114 specifically recognizes this MARUbe and extends it with K11-linked polyubiquitin chains. Using biochemical assays, siRNA-mediated

knockdowns, and structural modeling, we found that DTX2 loss abolishes MARUbe formation, whereas RNF114 depletion prevents K11-chain extension.

To probe this mechanism, we developed a chemoenzymatic method to generate a fluorescent Ub-ADPr conjugate (Ub-ADPrT) as a real-time probe for ligase activity. RNF114 preferentially acts on this substrate, catalyzing K11-linked chain extension validated by linkage-specific deubiquitylase profiling. AlphaFold3 modeling and mutagenesis identified a tandem Di19-UIM "M-UBD" (MARUbe-Binding Domain) within RNF114 that reads the ADPr mark and positions ubiquitin for K11 extension.

Comparative analyses revealed that RNF166 shares this M-UBD architecture and activity, defining a small family of "MARUbe-Targeted Ligases" (M-UTLs). Our findings establish RNF114 and RNF166 as reader-writer E3 ligases linking ADP-ribosylation to K11 polyubiquitylation, revealing MARUbylation as a multilayered PTM hub connecting PARP and ubiquitin signaling.

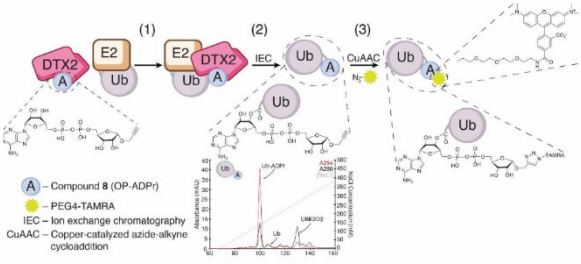
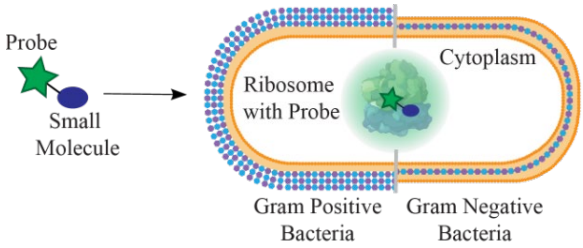


Figure: Schematic of the workflow for generating the fluorescent Ub-ADPrT probe, with a representative chromatogram showing its isolation by ion-exchange chromatography.

#9 - Development of Ribosomal RNA-Targeted Fluorescent Probes for Imaging and Permeability Studies in Bacteria

Ms. Meghan Rainier, University of Utah
Dr. Ming Chen Hammond, University of Utah



Rising antibiotic resistance is a global health threat that calls for innovative new strategies for the development and evaluation of next-generation antibiotics. A key challenge in the development of new antibiotics is the ability to accurately measure the cytoplasmic accumulation of novel antibiotics in bacterial cells. Current methods require genetic modifications (e.g., RNA aptamers and SNAP-tags), permanently fluorescent dyes, or labor-intensive techniques such as LC-MS, which lack single-cell, spatiotemporal, and live-cell resolution and are often slow and prone to variability. To overcome this, we have developed a cell-permeable fluorogenic probe that does not require genetic modification and is selectively activated by endogenous ribosomal RNA (rRNA) in live and fixed bacteria. This fluorogenic probe enables rapid labeling in *E. coli* and *B. subtilis* without the need for genetic engineering or wash steps. We have developed a site within the probe that minimally disrupts probe-rRNA interactions when conjugated to small molecules. By attaching the probe to an antibiotic, we have demonstrated its ability to track antibiotic permeability into the cytoplasm. We also identified a possible rRNA binding site for the probe. Currently, we are exploring the permeability mechanisms governing the entry of both free and antibiotic-conjugated probes into bacterial cells using Tn-Seq. This work offers a new approach to studying small-molecule permeability through rRNA interactions in live bacteria.

#10 - Oxidation state and ligand effects on nucleolar response pathways by Pt(IV) compounds

Christopher Griffin, University of Oregon
Prof. Victoria DeRose, University of Oregon

Small-molecule platinum drugs like cisplatin, oxaliplatin, and carboplatin have been widely

studied for their anti-cancer properties, particularly their ability to trigger cancer cell death by binding to nuclear DNA. While these compounds were initially believed to act through the DNA damage response (DDR) pathway, it has been demonstrated that oxaliplatin works via a unique nucleolar stress pathway, triggered by disruptions in the nucleolus, where ribosome biogenesis occurs. Studies have shown that tuning the non-labile ligands of platinum drugs, especially those bearing the diaminocyclohexane (DACH) ligand, affects their ability to induce nucleolar stress. Although effective, Pt(II) drugs are limited by severe side effects due to interactions with biological nucleophiles. To address this, Pt(IV) complexes, which are more kinetically inert, have been explored. Pt(IV) compounds, with their octahedral d6 geometry, are activated in cells by reduction to Pt(II), releasing axial ligands that modulate the drugs bioactivity. Our study explores the influence of both ligands and oxidation states on the ability of platinum compounds to induce nucleolar stress. By examining dicarboxylate Pt(IV) complexes derived from cisplatin and DACH-Pt scaffolds, we investigate how structural modifications impact their biological effects. These findings highlight the potential of Pt(IV) compounds in furthering the understanding of nucleolar stress pathways and their broader applications in cancer treatment.

#11 - Fluorogenic Antibiotic Probes for the Rapid Detection of Antibiotic Resistance

Dr. Sepehr Sebghati, University of Utah, Salt Lake City
Prof. Ming Chen Hammond, University of Utah, Salt Lake City

Antimicrobial resistance (AMR) is a growing global health crisis, projected to cause 10 million deaths annually by 2050. Despite the increasing prevalence of infections caused by antibiotic-resistant pathogens, standard methods used to diagnose resistance remain slow. Gold-standard phenotypic approaches rely on culturing bacteria in the presence of the drug for at least 16 hours. This delay forces clinicians to prescribe antibiotics empirically, before confirming whether they will be effective. When the infection is caused by a resistant bacterium, such treatment can lead to treatment failure and prolonged illness. Moreover, the widespread empirical use of antibiotics accelerates the spread of resistance by unnecessarily exposing bacteria to inappropriate drugs. To address this diagnostic gap, we developed a novel fluorogenic assay for the rapid identification of antibiotic-resistant bacteria. The assay utilizes fluorogenic antibiotic probes that light up when the antibiotic successfully binds its bacterial target, providing a quantitative, rapid readout of antibiotic activity and allowing resistant and susceptible bacteria to be distinguished within minutes. These probes are selective for the antibiotic targets and can identify resistance from both target site mutations as well as efflux pump activity, two of the main mechanisms of resistance. Collectively, our results reveal the potential of fluorogenic antibiotics for the rapid diagnosis of antibiotic resistance.

#12 AMEGSS: A Modular Platform for Rapid Generation of Stable GCE Cell Lines for Live-Cell Protein Labeling

Alex Eddins, Ph.D., Oregon State University

Genetic code expansion (GCE) enables the site-specific incorporation of noncanonical amino acids (ncAAs) into proteins, offering powerful tools for protein engineering and live-cell labeling. However, stable mammalian GCE systems remain challenging to implement, particularly for bioorthogonal labeling of intracellular proteins. Here, we present MEGSS (Modular Eukaryotic GCE Stable System) consisting of a single-plasmid, piggyBac-based platform for rapidly generating customizable, GCE-compatible stable mammalian cell lines. MEGSS allows modular assembly of synthetase/tRNA pairs, accessory genes, and reporters into a single integrable construct. We developed a “doubly silent” reporter system by combining TAG-interruption with doxycycline-inducible expression to enable precise control of fluorescent protein expression during cell line development. From this system, we derived TetReady, a clonal HEK293T cell line optimized for the incorporation of tetrazine-containing ncAAs. TetReady supports robust expression of both stably integrated and transiently introduced TAG-interrupted proteins. Using the fast and bioorthogonal sTCO–tetrazine ligation, we achieved rapid, specific, and low-background intracellular protein labeling in living cells. As a proof-of-concept, we visualized Akt-PH domain

translocation upon EGF stimulation, demonstrating the platform's utility for imaging dynamic protein localization. Together, MEGSS and TetReady offer a scalable and modular solution for GCE cell line development and live-cell bioorthogonal protein labeling, expanding the utility of ncAA technologies in mammalian systems.

#13 - Leveraging the dTAG system to study oncoprotein degradation in cells and mice

Dr. Saurav Kumar,# Fred Hutchinson Cancer Center, Seattle, Washington, USA.
Dr. Dezhi Li,# Laura and Isaac Perlmutter Cancer Center, New York University Langone Health, New York, New York, USA.
Dr. Ke Geng,# Laura and Isaac Perlmutter Cancer Center, New York University Langone Health, New York, New York, USA.
Dr. Yuan Hao,# New York University Grossman School of Medicine, New York, New York, USA._
Dr. Jiajia Gu,# Hillman Cancer Center, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA.
Dr. Annabel T. Olson, Fred Hutchinson Cancer Center, Seattle, Washington, USA.
Ms. Christina C. Kuismi, Fred Hutchinson Cancer Center, Seattle, Washington, USA.
Ms. Hye Mi Kim, Hillman Cancer Center, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA.
Dr. Yuanwang Pan, Laura and Isaac Perlmutter Cancer Center, New York University Langone Health, New York, New York, USA.
Ms. Fiona Sherman, Laura and Isaac Perlmutter Cancer Center, New York University Langone Health, New York, New York, USA.
Dr. Asia M. Williams, Hillman Cancer Center, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA.
Ms. Cassandra Thakurdin, Laura and Isaac Perlmutter Cancer Center, New York University Langone Health, New York, New York, USA.
Dr. Michela Ranieri, Laura and Isaac Perlmutter Cancer Center, New York University Langone Health, New York, New York, USA.
Ms. Mary Meynardie, Laura and Isaac Perlmutter Cancer Center, New York University Langone Health, New York, New York, USA.
Dr. Daniel S. Levin, Laura and Isaac Perlmutter Cancer Center, New York University Langone Health, New York, New York, USA.
Ms. Janaye Stephens, Laura and Isaac Perlmutter Cancer Center, New York University Langone Health, New York, New York, USA.
Ms. Alison Chafitz, Laura and Isaac Perlmutter Cancer Center, New York University Langone Health, New York, New York, USA.
Ms. Joy Chen, Fred Hutchinson Cancer Center, Seattle, Washington, USA.
Ms. Mia S. Donald-Paladino, Fred Hutchinson Cancer Center, Seattle, Washington, USA.
Mr. Jaylen M. Powell, Laura and Isaac Perlmutter Cancer Center, New York University Langone Health, New York, New York, USA.
Ms. Magdalena Ploszaj, Laura and Isaac Perlmutter Cancer Center, New York University Langone Health, New York, New York, USA.
Mr. Han Han, Laura and Isaac Perlmutter Cancer Center, New York University Langone Health, New York, New York, USA.
Dr. Tinghu Zhang, Stanford Cancer Institute, Stanford School of Medicine, Stanford University, Stanford, California, USA.
Dr. Medard Ernest Kaiza, Hillman Cancer Center, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA.
Dr. Alice H. Berger, Fred Hutchinson Cancer Center, Seattle, Washington, USA.
Dr. Tullia C. Bruno, Hillman Cancer Center, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA.
Dr. Nathanael S. Gray, Stanford Cancer Institute, Stanford School of Medicine, Stanford University, Stanford, California, USA.
Dr. Kwok-Kin Wong,*Laura and Isaac Perlmutter Cancer Center, New York University Langone Health, New York, New York, USA.
Dr. Hua Zhang,* Hillman Cancer Center, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA.
Dr. Behnam Nabet,* Fred Hutchinson Cancer Center, Seattle, Washington, USA.
#Equal contribution; *Corresponding author

Small molecule degraders, including PROTACs, that co-opt the cellular ubiquitin-proteasome system to deplete target protein levels and abrogate their function, represent a powerful approach in drug discovery. Despite their therapeutic potential, widespread applications of degrader are constrained by several factors including the necessity of a ligand that binds the target protein. Prior to investing in the discovery of selective ligands for degrader development, we previously pioneered a universal tag-based degradation technology known as the dTAG system. In this approach, we can effectively model the consequences of rapid and selective FKBP12(F36V)-tagged target protein loss using degraders known as dTAG molecules. In this study, we aimed to advance our strategy to studying the pharmacological consequences of

targeted degradation of cancer drivers in immune-competent mouse models. To achieve this goal, we focused on KRAS, the most frequently mutated oncogene in pancreatic and lung cancers. While breakthrough drug discovery approaches have significantly advanced the clinical management of KRAS G12C patients, several mutations including KRAS G12V still lack selective small molecules. We focused on evaluating the immediate and prolonged consequences of targeted KRAS G12V degradation in vitro and in vivo using the dTAG system. By developing several cellular models, we first confirmed the functionality of FKBP12(F36V)-KRAS G12V, demonstrating that KRAS G12V and FKBP12(F36V)-KRAS G12V similarly activated downstream signaling, transformed cells, and established tumors in mice. Treatment with dTAG molecules, completely reversed these responses, collapsing downstream signaling and abrogating tumor growth. Next, we established the first oncogene-induced transgenic dTAG mouse models. We developed mouse models to conditionally activate FKBP12(F36V)-KRAS G12V in the lung epithelium, leading to the robust development of lung adenocarcinoma. Administration of dTAG molecules significantly reduced downstream signaling and led to robust tumor regression. Strikingly, we observed that KRAS G12V degradation induced a pro-inflammatory cytokine response and reprogrammed the tumor microenvironment. This led to a robust antitumor immune response characterized by enhanced effector and cytotoxic $CD8^+$ T cell infiltration. Together, this work illustrates the versatility of the dTAG system for pre-clinical target validation in both cellular and immune-competent mouse models. Our findings demonstrate the pronounced tumor-intrinsic and tumor-extrinsic impacts upon mutant KRAS degradation and provide strong evidence for the clinical potential of targeted degradation of mutant KRAS in lung and pancreatic cancers.

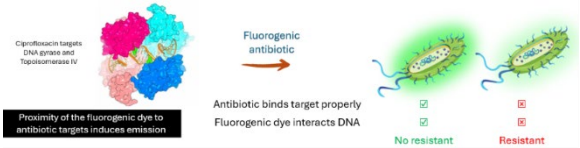
#14 - Fluorogenic antibiotics as activity-based probes to evaluate antibiotic resistance in bacteria

Dr. Cinthia Laura Hernandez Juarez, University of Utah
Prof. Ming Chen Hammond, University of Utah

Antibiotic treatments have progressively lost their efficacy in treating bacterial infections because of the growing problem of antibacterial resistance. This resistance occurs as a natural evolutionary process in bacteria, but the excessive and inadequate use of antibiotics leads to the wide spread of resistant bacteria and their antibiotic-resistant genes. The CDC estimates that the use of 50% of antibiotics in hospitals is inappropriate or unnecessary. So, quick diagnostic tests that detect drug-resistant bacteria can help direct appropriate treatment approaches and limit the spread of resistance. Nowadays, existing phenotypic and genotypic techniques for detecting antibacterial resistance are constrained by limitations in sensitivity, processing time, and high cost.

Here in we develop a low time-consuming and real-time approach to detect antibiotic resistance in bacteria using a novelty class of probes called fluorogenic antibiotics. These probes consist of a derivative of fluorogenic dye which is attached to the antibiotic ciprofloxacin through a linker. The inclusion of fluorogenic dye allows emission to be observed only when this probe fragment interacts with sites that appear to correspond DNA gyrase and topoisomerase IV, which are the targets of ciprofloxacin. In this context, fluorogenic antibiotics act as activity-based probes for these enzymes.

To optimize the fluorescence of our probe and expand the emission color palette, different probes with changes to the structures of dyes and linkers have been synthesized. Ongoing screening assays show that the photophysical properties of the probe can be improved and non-specific interactions in bacteria can be reduced with certain combinations of dye and linker structures. The probes show fluorescence response using flow cytometry experiments in Gram-positive bacteria *B. subtilis* and Gram-negative bacteria *E. coli* without wash steps. The application of fluorogenic antibiotics as activity-based probes could be a powerful tool to diagnose antibacterial resistance and visualize enzymatic activity of bacteria in real-time.



#15 - Sequencing-Based Recorders for Multiplexed Analysis of Kinase Signaling Activity in Cells

Doria Unrau, University of Washington
Dr. Dustin J. Maly, University of Washington

Cellular signaling networks control how cells sense and respond to stimuli, regulating processes like growth, metabolism, and gene expression. A central feature of these networks is the dynamic regulation of kinase activity. Although multiple signaling pathways converge to control cellular state, systematic measurement of kinase activity across many pathways remains limited. Current tools such as antibodies and fluorescent biosensors provide valuable insight but are restricted by specificity, scalability, and multiplexing capacity. To address these limitations, we are developing high-throughput sequencing (HTS)–compatible molecular recorders that capture kinase activity as stable molecular tags. These recorders detect transient phosphorylation events and convert them into enrichable marks, enabling their capture and quantification by sequencing. The system uses circular RNA barcodes containing an MS2 hairpin that binds the MS2 coat protein (MCP) with high specificity. Fusing MCP to a protein of interest enables phosphorylation-dependent tagging, selective enrichment of associated barcodes, and sequencing-based readout of kinase activity. We engineered genetically encoded intracellular reporters that record phosphorylation of kinase-specific peptide substrates as enrichable molecular tags. As a proof of concept, we generated Protein Kinase A (PKA) recorders by coupling phosphorylation-dependent FHA1 binding domains to molecular recorder architectures such as cpHalo or biotin ligases. Using pharmacological activation and inhibition of PKA, we validated that these recorders accurately distinguish stimulated and inhibited states and generate reproducible barcode enrichment profiles. This work establishes a validated workflow for kinase activity recording using sequencing-based readouts. The modular design allows systematic substitution of kinase-specific substrates, creating a scalable toolkit for diverse signaling pathways. These recorders will enable massively parallel studies of signaling networks, functional consequences of protein variants, and pharmacological perturbations. Ultimately, this platform provides a broadly applicable toolkit for dissecting how dynamic biochemical activities shape cellular function.

#16 - Redox regulation of host ribosomal proteins during Helicobacter pylori infection

Ms. Renuka Ramanathan, Yale University
Prof. Stavroula Hatzios, Yale University

Infection with the gastric pathogen Helicobacter pylori contributes to the development of severe gastric pathologies, including gastritis, peptic ulcer disease, and gastric adenocarcinoma. H. pylori has evolved various mechanisms to evade the host immune response, enabling the pathogen to persist for decades in the gastric mucosa. Chronic infection promotes the continuous generation of reactive oxygen species (ROS) by neutrophils and gastric epithelial cells. Elevated levels of ROS can damage macromolecules, but beyond oxidative DNA damage, little is known about other cellular targets of oxidative stress during H. pylori infection. Proteins containing redox-sensitive cysteines are particularly susceptible to oxidative modifications that can alter protein structure, function, or localization. To identify host protein targets of oxidation during infection and understand the resulting impacts on host physiology, we performed a chemical proteomic screen in human gastric cancer cells infected with H. pylori using reactive cysteine profiling. Among the top hits were cysteines from several ribosomal proteins. Since ribosomes are essential for protein synthesis and cell growth, and host translation is known to be inhibited by other pathogens, we hypothesize that H. pylori infection perturbs host ribosome function. Using a combination of polysome profiling and translation assays, we identified infection-induced changes in host protein synthesis and ribosome assembly. In parallel, we discovered that the abundance and distribution of key factors involved in translational control are altered in H. pylori-infected cells. We are currently using Cryo-EM and chemical proteomic analyses to investigate the molecular basis for these translational defects and assess whether infection-induced changes in cysteine reactivity shape ribosome assembly. Together, these studies aim to provide mechanistic insights into how H. pylori modulates host ribosome-

associated processes and expand our understanding of the host translational landscape during infection.

#17 - Targeted Degradation of Oncogenic TRK Fusion Proteins

Mia S. Donald-Paladino, Fred Hutchinson Cancer Center
Dr. Saurav Kumar, Fred Hutchinson Cancer Center
Dr. Jiewei Jiang, University of California, San Diego
Joy Chen, Fred Hutchinson Cancer Center
Andrea Gutierrez, Talus Bioscience
Dr. Alexander J. Federation, Talus Bioscience
Dr. Frank Szulzewsky, University of Utah
Prof. Eric C. Holland, Fred Hutchinson Cancer Center
Prof. Fleur M. Ferguson, University of California, San Diego
Prof. Behnam Nabet, Fred Hutchinson Cancer Center

Chimeric fusion proteins formed by chromosomal translocations involving tropomyosin receptor kinase (TRK) and various partner proteins have been identified as oncogenic drivers in several adult and pediatric cancers. In normal development, TRK proteins are primarily expressed in neurons. Their activity is regulated by ligand-induced oligomerization, which triggers downstream signaling essential for neuronal function and the maintenance of neural networks. In cancer, TRK fusion partners often contain oligomerization domains which facilitate constitutive activation of TRK kinase activity and oncogenic downstream signaling. Targeting TRK fusions in cancer is an active area of drug discovery efforts. First-generation TRK inhibitors, including entrectinib and larotrectinib, have shown positive responses in TRK fusion-positive cancers. However, their therapeutic potential is limited by off-target effects and the emergence of resistance. Although second-generation TRK inhibitors are currently under clinical investigation, there remains a critical need for sustained and selective therapeutic strategies to suppress TRK-driven oncogenic signaling. To address this, we developed heterobifunctional small-molecule degraders (PROTACs) that recruit cereblon to achieve complete elimination of TRK fusion proteins. We identified JWJ-01-378, which rapidly (< 1 h) and potently (< 10 nM) degrades TRK fusions including TPM3-TRKA. We further show that JWJ-01-378 induces TPM3-TRKA degradation through the ubiquitin-proteasome system, and confirm the acute selectivity of JWJ-01-378 through proteomics analysis. Importantly, TPM3-TRKA degradation by JWJ-01-378 suppresses downstream signaling and reduces cancer cell viability, with improved responses compared to heterobifunctional control compounds that cannot degrade TPM3-TRKA. Together, our study describes the development of a TRK fusion-targeting PROTAC which will serve as a valuable chemical probe for interrogating TRK fusion biology and evaluating targeted degradation as a therapeutic approach. In continued work, we will evaluate the use of JWJ-01-378 in degrading diverse TRK fusions, study the mechanisms by which TRK fusions dysregulate signaling, and develop approaches that overcome resistance to current clinical candidates.

#18 - Small-molecule ligand screening of the Chlamydia trachomatis type III secretion system chaperone complex, Scc4:Scc1

Ms. Isabella A. Kibler, John Carroll University
Dr. Megan A. Macnaughtan, John Carroll University

In 2024, over 1.5 million cases of Chlamydia were reported in the United States. The heterodimer complex, Scc4:Scc1, is a critical protein in Chlamydia trachomatis. Scc4:Scc1 is a type III secretion system chaperone for the essential virulence factor, CopN. To search for small compounds that bind Scc4:Scc1 and prevent CopN secretion, Scc4:Scc1 was screened against a protein-protein interactions library of 6,080 compounds using the thermal shift assay (TSA). The TSA produced a melt curve, and the melting temperature of the protein was determined using the derivative method. Changes to the melting temperature were used to identify compounds that bind to Scc4:Scc1. The binding was confirmed by additional TSA experiments to test reproducibility and concentration dependence. Several compounds were identified as reproducible hits. Docking and molecular dynamics simulations were used to model the binding of the hit compounds to Scc4:Scc1. Future studies of these interactions will include nuclear magnetic resonance spectroscopy and isothermal calorimetry.

#19 - Discovery of a common chemical scaffold targeting the Chlamydia

trachomatis type III secretion chaperone complex, Scc4:Scc1

Ms. Felicity M. Kolb
Dr. Megan A. Macnaughtan

Chlamydia trachomatis is responsible for the most prevalent bacterial sexually transmitted infection. The protein, Scc4, has critical roles in transcription when it binds RNA polymerase and in the bacterium's type III secretion system when it binds Scc1 to chaperone the essential virulence factor, CopN. The important dual-function and unique properties of this protein make it a promising target for drug discovery. Protein-ligand interactions were examined utilizing the thermal shift assay; 1,280 compounds were tested in mixtures of four as well as individually for binding to the chaperone complex, Scc4:Scc1. Ligands that produced a significant shift in the melting temperature indicated a conformational change in the complex that stabilized or destabilized the protein. A common chemical scaffold was identified that destabilized the complex. Reproducible protein-ligand interactions were further investigated using docking and molecular dynamics simulations.

#20 - Small-molecule ligand screening of the dynamic Chlamydia trachomatis RNA polymerase-binding protein, Scc4

Ms. Margaret H. Fuller, John Carroll University
Dr. Megan A. Macnaughtan, John Carroll University

The most common sexually transmitted bacterial infection, Chlamydia, is caused by the bacterial pathogen Chlamydia trachomatis (Ct). This bacterium utilizes a type three secretion system (T3SS) to transport virulence factors into host cells. The T3SS chaperone complex of proteins Scc1 and Scc4 stabilizes the virulence factor, CopN, an essential protein for Ct pathogenesis. Scc4 is unique as it functions as a part of the T3SS during the late and early states of infection and binds RNA polymerase during the mid-stage of infection altering transcription. The bifunctional nature of Scc4 enables it to serve as a dual target for drug discovery. A thermal shift assay was used to screen the Scc4:Scc1 complex and Scc4 for binding to a library of 6,080 compounds known to disrupt proteins. Ligands identified to shift the melting temperature of the complex indicated binding and were selected as preliminary hits. Because the structure of Scc4 is dynamic, it does not produce a typical melt curve. Ligand binding to Scc4 is identified by deviations from the melt curve produced by the protein alone. Ligands identified as preliminary hits are validated with concentration dependent assays. Further investigation of specific hit-protein interactions will include ligand docking and molecular dynamics simulations, nuclear magnetic resonance spectroscopy, and isothermal calorimetry.

#21 - Mapping Long Range Allosteric Networks Governing KRAS Pocket formation using LABEL-Seq

Aman Shihora
A. Felicia Adebanjo
Dr. Jessica Simon
Allyssa Vandi
Dr. Raining Wang
Melinda K. Wheelock
Jordan Opsahl
Dr. Douglas M. Fowler
Dr. Dustin J. Maly

Small GTPases are membrane-bound molecular switches that act as central signaling hubs controlling diverse cellular processes. Among these, KRAS is one of the most frequently mutated oncogenes in human cancer. For decades, KRAS was deemed undruggable due to its lack of a targetable, deep binding pocket typically targeted by small molecules. The recent discovery of cryptic allosteric pockets has enabled the development of two mechanistic classes of inhibitors: allosteric modulators and molecular glues. While structural studies have defined key binding residues, the long range allosteric networks that dictate pocket formation remain poorly understood. To systematically map these networks, we leverage LABEL-seq, a sequencing based assay that allows for the multiplexed functional readout of thousands of single point KRAS variants. This approach enables us to quantify the contribution of every amino acid on the stability of the inhibitor bound state and how this translates to downstream signaling. Using this framework, we defined the molecular determinants that enable AMG-410, an allosteric pan-KRAS inhibitor, to engage its pocket across many amino acid substitutions. We were able to map vital binding pocket residues and identify distal mutations that

modulate conformational favorability for inhibitor engagement. A parallel LABEL-seq analysis with RMC-6236, a Cyclophillin-A mediated molecular glue, showed that variations at the CypA-KRAS interface divergently influence inhibitor sensitivity. We further demonstrate that activation of KRAS alters its conformational landscape for inhibitor binding, invoking distinct allosteric networks that alter inhibitor binding. Together, these findings highlight the complex distal interactions that govern ligand binding and how nucleotide state impacts adoption of these states. Ongoing work aims to reveal how stability of the ligand-bound conformation influences effector interactions, providing mechanistic insight into inhibitors reshaping signaling conformations.

#22 - Elucidating the Mechanism of Formation of 7-Amido-7-Deazaguanine in DNA by the Enzyme DpdC

Mr. Andrew Buckley, Portland State University
Mr. Jonathan Xu, Case Western Reserve University
Dr. Dirk Iwata-Reuyl, Portland State University

Nucleic acid modification research has identified over 200 DNA and RNA modifications with a wide range of chemical complexity and roles at various levels of physiology. Among the modifications found in both DNA and RNA are members of the 7-deazaguanine family of nucleic acid modifications. In RNA, the 7-deazaguanine derivatives queuosine and archaeosine have roles in regulating translation efficiency and tRNA structural stability. In DNA, the 7-deazaguanine derivatives 7-cyano-7-deazaguanine (preQ0) and 7-amido-7-deazaguanine (ADG) are components of an elaborate bacterial restriction-modification system known as the Dpd system, but the chemistry and structural components underlying the formation of these two modifications in DNA are largely uncharacterized. The Dpd system is comprised of a modification component, DpdA-C, and a restriction component, DpdD-K, with the precise roles of the proteins found in the restriction component remaining unidentified. In the modification component, a transglycosylase, DpdA, and an ATPase, DpdB, are responsible for the sequence-dependent substitution of guanine (G) with preQ0. After modification of DNA with preQ0, a nitrile hydratase, DpdC, converts preQ0-DNA to ADG-DNA. Research into the transglycosylation of G-DNA to preQ0-DNA has identified catalytic residues on DpdA, with a current focus on the role of DpdB as a putative ATP-dependent helicase. Less is known about the DpdC-catalyzed hydration of preQ0-DNA to ADG-DNA. Described here are the development of a quantitative assay for measuring DpdC activity and mutagenesis experiments that point to a putative active-site residue critical for catalysis. Immediate and future goals include further efforts in elucidating the DpdC reaction mechanism as well as the determination of the sequence-specificity and structure of DpdC. Results from this work will broaden our understanding of restriction-modification systems and will expand on what is known about the biochemical transformations of nitriles.

#23 - Preparation of Isolated Human Pancreatic Islets for Cryo-electron Tomography

Andrew Gustafson, OHSU
Dr. Carsten Schultz, OHSU
Dr. Benjamin Barad, OHSU

Pancreatic islets, or islets of Langerhans, are specialized clusters of endocrine cells that produce the hormones necessary to maintain glucose homeostasis. While the isolation of the islets from the pancreas does remove some biologically relevant context, notably the islet's vasculature, islets retain their secretory abilities and cell-cell connectivity, and isolated islets are used in human pancreatic islet transplants to treat diabetes. Imaging isolated islets by cryo-electron tomography (cryo-ET) would offer unprecedented detail of cellular ultrastructure and islet architecture under biologically relevant conditions. However, preparing tissue of this type for cryo-ET presents significant challenges, including how to vitreously freeze islets on electron microscopy grids, how to stain or label whole islets or specific cell-types for identification, and how to thin the frozen sample for transmission electron microscopy. Here we detail our efforts to overcome these challenges using high-pressure freezing along with correlative light and electron microscopy (CLEM) followed by focused ion-beam milling. Despite no established methods for handling isolated islets for this type of workflow, we have made promising progress that we hope will be the basis for future investigations that will help us

study phenomenon like the dynamic rearrangement of the plasma membrane and cytoskeleton at the location of insulin secretion, as well as reveal details of cell-cell connections between the multiple cell-types in islets.

#24 - Phosphoinositides in pancreatic adenocarcinoma

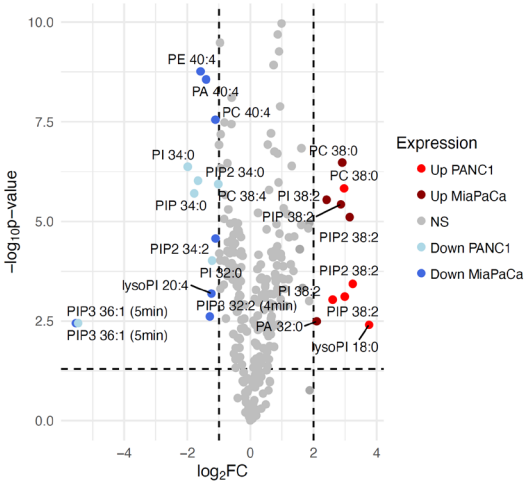
Dr. Berit Blume, Oregon Heath & Science University
Dr. Jacob Porter, Oregon Heath & Science University
Mrs. Grace Potter, Oregon Heath & Science University
Dr. Dove Keith, Oregon Heath & Science University
Dr. Jonathan Brody, Oregon Heath & Science University
Dr. Alexis Traynor-Kaplan, ATK Analytics Innovation and Discovery
Dr. Andrew Emili, Oregon Heath & Science University
Dr. Carsten Schultz, Oregon Heath & Science University

Lipids and lipid metabolism play a crucial role in pancreatic ductal adenocarcinoma (PDAC) tumor progression, influencing cell signaling, tumor metabolism, and therapeutic resistance (Yin et al., 2022). Pancreatic ductal adenocarcinoma (PDAC) is among the deadliest cancers, with a five-year survival rate below 10 percent (Miller, 2016). New approaches are urgently needed, since most therapies fail due to late detection and the disease's ability to rewire its biology and resist treatment. Identifying biomarkers for early detection as well as new treatment methods would significantly improve outcomes for patients.

Our early work using high-resolution lipidomics on two pancreatic cancer cell lines (PANC1 and MiaPaCa2) showed strikingly up- and down-regulated phosphatidylinositol lipids compared to immortalized healthy cells (HPNE) (Figure 1a). Our lipidomic data on PDAC patient bulk tissues revealed striking shifts in lipid species compared to the healthy pancreas, highlighting the biological and clinical importance of tumor lipid remodeling (Figure 1b). We hypothesize that these remodeled lipid networks act as critical drivers of disease progression and resistance. Understanding them could expose new therapeutic targets that have been overlooked by genetic and protein network research.

Our preliminary deep proteomic analysis, generated on the same cell samples in parallel, revealed PIK3CB downregulation in both MiaPaCa2 and PANC1 cell lines, suggesting disruption of PI3K signaling in PDAC (Figure 2). Conversely, several lipid-handling enzymes were significantly elevated including CERS2, CPTP, ELOVL5, and ORMDL3, while other differential proteins have prognostic significance in PDAC (Human Protein Atlas), including ANXA6, PIK3CB, JAG1, INPP5K, SERPINE1, LDAH, and OSBPL10. Notably, DGKE is of particular interest due to its role in diacylglycerol metabolism within the phosphatidylinositol turnover cycle, which directly impacts lipid signaling pathways relevant to PDAC progression.

We are currently exploring both plasma samples for early detection and the impact of the tumor microenvironment on our tissue results. As a next step we will use our multifunctional phosphoinositides to explore the protein interactions of identified upregulated lipids.



A.

B

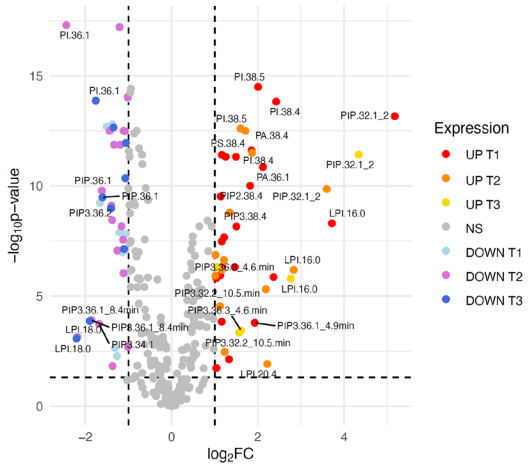


Figure1: Volcano plots of lipidomic analysis of PDAC samples. A. Volcano plot of lipidomic analysis of two PDAC cell lines (PANC1, MiaPaCa2) highlighting significantly altered species compared to an immortalized pancreatic duct cell line (HPNE). B. Volcano plot of lipidomic analysis of human pancreatic tumor tissue highlighting significantly altered species compared to human healthy pancreatic tissue.

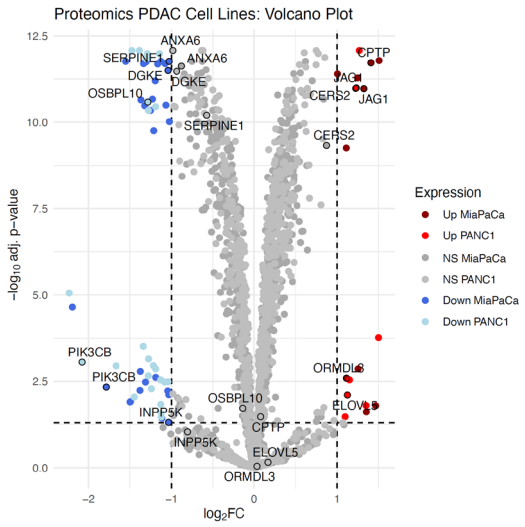


Figure2: Volcano plot showing significantly differential proteins identified by quantitative proteomics analysis on the same tumor (PANC1, MiaPaCa2) and pancreatic duct (HPNE) cell line samples.

#25 - Structural basis of Mg-nucleotide regulation and the role of intrinsically disordered regions on vascular Kir6.1/SUR2B KATP channels

Dr. Camden M. Driggers, Oregon Health & Science University
Dr. Katarzyna Walczewska-Szewc, Nicolaus Copernicus University
Dr. Sun-Joo Lee, Washington University in St. Louis
Dr. Yi-Ying Kuo, Oregon Health & Science University
Ms. Zhongying Yang, Oregon Health & Science University
Prof. Colin G. Nichols, Washington University in St. Louis
Prof. Show-Ling Shyng, Oregon Health & Science University

Vascular KATP channels comprising pore-forming Kir6.1 and regulatory SUR2B subunits regulate blood flow and blood pressure by modulating vascular tone. Variants with increased activity result in severe cardiovascular pathologies known as Cantú syndrome. Unique among KATP isoforms, vascular KATP channels lack spontaneous activity and require Mg-nucleotides for activation, but once activated, they are relatively insensitive to ATP inhibition. To gain insights into the structural basis of their nucleotide regulation, we determined cryoEM structures of vascular KATP channels in the presence of MgATP and MgADP. From a single cryoEM sample we obtained distinct conformations of the fully-assembled channel, including SUR2B in a MgADP-free nucleotide binding domain (NBD)-separated state, a MgADP-bound NBD-dimerized state, and a range of intermediate conformations defining the structural transition. These structures reveal the cryoEM densities of intrinsically disordered regions (IDRs) of both Kir6.1 and SUR2B with functional implications. In the NBD-separated conformation, the Kir6.1-N terminus (KNt) is stabilized within the central cleft of SUR2B's ABC-core. In contrast, in the NBD-dimerized conformation, KNt is excluded from the central cleft; instead, it is held by an ED domain consisting of 15 consecutive glutamate and aspartate residues within SUR2B's N1-T2 linker which connects NBD1 (N1) with transmembrane domain 2 (T2). Notably, a regulatory helix in the N1-T2 linker is wedged between the two NBDs in the NBD-separated conformation, but upon NBD-dimerization it moves outside the NBDs and interacts with the 42 C-terminal residues that are unique to SUR2B. All-atom molecular dynamics simulations of the full channel

captured conformation-specific interactions between SUR2B's N1-T2 linker, KNt and the Kir6.1 C-terminus. Our structures and the dynamic interplays observed between the IDRs suggest a crucial role of IDRs in MgADP/MgATP-dependent conformational switch in vascular KATP channels.

#26 - Pyrylium Ring Editing Chemistry Enables Selective Detection of Ammonia in Cells

Dr. Cheng Yang, Princeton University
Logan Tenney, Princeton University
Prof. Christopher J. Chang, Princeton University

Ammonia is one of the simplest bioactive molecules, but essential for all life forms as a fundamental building block for the biosynthesis of amino acids, proteins, and nucleic acids. Despite its significant role, chemical tools for selective ammonia detection within biological specimens remain limited due to the chemoselectivity between ammonia and all other biologically related amines. In our study, we aim to utilize pyrylium ring editing chemistry to develop an activity-based fluorescent probe that can selectively detect ammonia, with a long-term goal of using such compounds for deciphering roles of ammonia in cellular nitrogen cycle. By attaching pyrylium reactive group to a boron-dipyrromethene (BODIPY) fluorophore, we developed a photo-induced electron transfer (PeT)-based fluorescent probe. In-vitro study suggests that only ammonia can provide a fluorescence turn-on response among other amino acids. Furthermore, our probe is able to detect ammonia in fixed cells at a millimolar level.

#27

#28 - Unusual base triple determines tRNA substrate specificity for universal t6A modification

Mr. Erik Scheleen, Portland State University
Mrs. Ana Kutchuashvili, San Diego State University
Dr. Manal Swairjo, San Diego State University
Dr. Dirk Iwata-Reuyl, Portland State University

The universal N6-threonylcarbamoyl adenosine (t6A) tRNA modification at position 37 of ANN (where N is A, U, G, or C) decoding tRNA is necessary for translational fidelity. In bacteria, the biosynthesis of t6A modified tRNA begins with the formation of an intermediate, threonylcarbamoyl-AMP (TC-AMP), by TsaC/TsaC2. A complex comprised of two proteins, TsaB and TsaD, known as the threonylcarbamoyl transferase (TCT) complex, then transfers the threonylcarbamoyl moiety from TC-AMP to A37 of substrate tRNA, and an additional protein, TsaE, appears to reset the catalytic complex. Substrate tRNA have a strictly conserved 36-UAA-38 sequence, but the basis for specificity derived from this sequence has remained unclear. Here, we report mutagenesis data supporting a 3.7 Å cryo-EM structure of substrate tRNA bound to Thermotoga maritima TsaB2D2 with unmodified A37 in the active site. The bound tRNA adopts a strained anticodon loop conformation with U36, A38, and U32 forming a base triple providing a mode of indirect readout for substrate recognition. Non-specific contacts between the complex and the D-stem of the tRNA provide an additional mode of indirect readout. Site-directed mutagenesis corroborates the structural data with mutated residues interacting with the base triple resulting in the greatest loss of activity whereas tRNA D-stem mutants demonstrated relatively minor changes in activity. Together, the structural and mutagenesis data elucidate the basis for the restriction of the modification to ANN decoding tRNA by the TCT complex and discrimination against non-ANN decoding tRNA.

#29 - A Targeted Chemoproteomic Platform for Structural Mapping of Proteins and Protein Variants

Daniel Brush, University of Washington
Dr. Shao-En Ong, University of Washington
Dr. Dustin J. Maly, University of Washington

Characterizing protein dynamics within their cellular context is essential for understanding protein conformational dynamics, allosteric regulation, and ligandability. Mass spectrometry (MS)-based methods have emerged as powerful tools for probing protein structure and conformational dynamics in heterogenous biological samples. However, there remains a limited set of chemical tools that can probe a broad range of proteins within their cellular environment and at sufficient resolution to enabling in-depth mapping of protein surfaces. Typical shotgun proteomic methods can profile

thousands of proteins simultaneously, but insufficient coverage precludes the ability to fully characterize a protein. Conversely, current high resolution structural methods can only study one or a few proteins at a time, limiting its throughput. Thus, approaches that can probe protein structure and dynamics across many proteins simultaneously while maintaining sufficient resolution are needed.

To address these limitations, we have developed a targeted proteomic platform for mapping protein dynamics in cells. Our method relies on generating pools of protein fusions, fusing each protein-of-interest with a selective and enrichable affinity tag. Protein fusions are then enriched from lysate and processed for LC-MS/MS detection of tryptic peptides. We show that combining our enrichment strategy with data-independent acquisition (DIA) yields a simpler sample matrix enabling moderate to high sequence coverage for each protein fusion. In parallel, we show that integrating DIA with Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) expands multiplexing capabilities without compromising quantification reproducibility or sequence coverage. Using our targeted DIA-SILAC platform, we are now exploring using cell-permeable electrophilic fragments to covalently react with nucleophilic amino acids and quantitatively measure engagement. We can then utilize site-specific residue modification as a proxy for monitoring structural changes for a specific protein. Together, these features enable protein surface mapping for many proteins simultaneously.

Given the amendable nature of our method, we focused on generating a pool of disease-relevant signaling proteins, consisting mainly of kinases and GTPases, along with a set of cancer-associated disease mutants. We will employ our targeted chemoproteomic platform to compare both the wild-type and disease-associated mutants to elucidate differences in their allosteric regulatory networks and identify possible ligandable sites. This work will offer greater mechanistic insight into how disease mutations reshape protein structure and function in cells.

#30 - Computational Design of a Chemically-Controlled Molecular Switch with Optimal Properties

Fernando Banales Mejia, University of Washington
Prof. Dustin J. Maly, University of Washington

Precise, modular, bioorthogonal tools are essential for controlling cellular processes. Here, we describe our efforts to computationally design an integrated, chemically induced dimerization (CID) and chemically disruptable proximity (CDP) system based on the HCV protease NS3a with optimal intracellular properties. NS3a is bound by an autoinhibitory partner (ANR) and modulated by approved HCV protease inhibitors, including Danoprevir—addressing two practical limits: suboptimal mammalian-cell expression of NS3a and partner affinity that constrains performance in demanding applications. To optimize intracellular expression levels, we applied ProteinMPNN to design stabilized NS3a variants that retain their ability to bind Danoprevir and dimerize with the dimerization partner DNCR2, a high-affinity binder to the NS3a:danoprevir complex. Across 21 designs, multiple variants demonstrated markedly improved cellular expression compared to a previously developed solubility-optimized NS3a, while retaining ligand-dependent binding, as verified by DNCR2-based enrichment assays in mammalian cells. To develop a CDP system with optimal affinity for chemical-genetic engineering applications, we computationally designed a library of minibinders that target the drug-binding interface of NS3a. I will describe the use of LABEL-Seq to profile intracellular engagement of each minibinder with a stability-optimized NS3a variant. The platform provides two readouts: barcode-abundance profiles that quantify engagement across the library, and ligand-dependent proximity labeling via a TurboID–stability-optimized NS3a fusion, with sequencing of enriched barcodes identifying the highest-affinity minibinders. Together, deep-learning-guided design and multiplexed functional screening enhance the NS3a-based CDP module into a higher-expression, higher-affinity state while preserving bioorthogonality, enabling control across diverse cellular systems.

#31 - Synthesizing Electron Donor and Acceptor [n]Cycloparaphenylenes (CPPs) for Biotechnology Applications

Gabriela M. Bailey, University of Oregon
Prof. Ramesh Jasti, University of Oregon

[n]Cycloparaphenylenes ([n]CPPs) are small molecular analogs of armchair carbon nanotubes with unique chemical and optical properties. The remarkable photophysical properties of [n]CPPs include a shared maximum absorbance around 340 nm, size-dependent emission (450–530 nm from [12]CPP to [8]CPP), and molar extinction coefficients on an order of magnitude of 104 or 105. The synthesis of [n]CPPs typically relies on bottom-up organic approaches that allow precise control over ring size and incorporation of different functional units, enabling the incorporation of water-solubilizing and organelle-targeting groups for biological purposes. Our study reporting a molecule with an organelle targeting group demonstrated that the compound is amenable to two-photon imaging, enabling deeper tissue penetration. These studies highlight the capabilities of these fluorophores as fluorescent tags for biological imaging with potential diagnostic utility. However, a previous limitation was the emission spectral range, which was limited to the blue/green region.

Recent work from our group and others has investigated the incorporation of electron donors and acceptors into or on the periphery of the [n]CPP scaffold. These modifications have broadened the palette of available emission colors for the scientific community. A recent study from our group specifically investigated how systematic, structural modifications via the incorporation of thiophene (electron donor) and benzothiadiazole (BT) to the [10]CPP and [12]CPP scaffold alter the photophysical properties. Seven novel electron donor-acceptor [n]CPPs (D-A[n]CPPs) were reported with emission wavelengths ranging from 469 – 639 nm. The quantum yields in DCM ranged from 0.33 to 0.06, and in DMSO from 0.22 to 0.03, highlighting their solvatofluorochromism properties. This study has provided fundamental knowledge on the synthetic design strategies to prepare D-A[n]CPPs with desired photophysical properties. Furthermore, the computational emission trends are consistent with the experimental results, indicating that these computational tools can be used to identify red-emitting D-A[n]CPPs within a series of proposed target molecules.

Expanding upon this work, our goal is to combine knowledge from the water-solubilizing and organelle-targeting group studies and the photophysical properties study to prepare water-soluble D-A[n]CPPs with orange and red emission. The target structures contain a TIPS-protected alkyne, which, once the final target molecule is synthesized, will be used as a functional handle in an alkyne-azide click reaction to append a PEG-linked NHS ester. Attachment of the NHS ester will allow for bioconjugation reactions to antibodies, which have not been reported before for [n]CPPs. Potential applications for these novel compounds include their use as fluorophores in multiplex imaging, as their shared absorbance maximum enables simultaneous excitation while they emit at different wavelengths of light.

#1 Visualization of white matter tissue during surgery with near-infrared, tissue-specific, small-molecule fluorophores.

Biruk Mengesha1, Antonio R. Montaño1, Mingchong Dai1, Stevie Huynh1,2, Summer L. Gibbs1,2, Lei G. Wang1,2

1Department of Biomedical Engineering Department, 2Knight Cancer Institute, Oregon Health & Science University, Portland, OR, 97201, USA

Surgical complications involving white matter tissue in which white matter tracts are damaged can cause declines in both cognitive and motor capability. A method of real-time visualization can provide the needed assistance to guide surgeons and improve white matter preservation while maximizing oncologic resection. Therefore, minimizing any risk of postoperative difficulties experienced by the patient and making recovery less cumbersome. The method of visualization as described above is not available in clinics, leading our group to develop near-infrared small molecule probes that can provide this white-gray matter delineation. These probes offer inherent white matter specificity without the use of a separate targeting motif (e.g., antibody or peptide), low molecular weights (i.e., <500 Da), and near infrared fluorescence, which when used in tandem with clinical imaging systems provide a means to discriminate white matter tissue from gray matter and cancerous tissue. In addition to these probes being well suited for fluorescence-guided surgery, when paired with tumor specific probes in a separate imaging window, two-color images are possible allowing for tumor-white matter differentiation to be improved dramatically. The versatility and functionality of these probes position them well for clinical applications and improvements in patient outcomes.

#2 - Innovating the first enzymatic tool to discover non-canonical ubiquitin esters

Dr. Rachel E Lacoursiere, Oregon Health & Science University
Dr. Daniel S Bejan, Oregon Health & Science University
Dr. Michael S Cohen, Oregon Health & Science University
Dr. Jonathan N Pruneda, Oregon Health & Science University

Every research field has its forgotten middle child: The part of the field that is inaccessible with conventional experiments and is begging for someone to create a tool, technique, or experiment to facilitate its advancement. In the ubiquitin field, the discovery of non-canonical substrates and linkages relies heavily on the design of new enzymatic tools. Canonically, ubiquitin covalently modifies protein substrates on target Lys residues; however, we and others have highlighted a diverse array of substrates including lipids, carbohydrates, and nucleic acids. Many of these non-protein substrates form an ester linkage to ubiquitin, increasing the difficulty of their study due to the labile nature of the conjugates. After identifying a bacterial deubiquitinase with dual canonical and non-canonical activity, TssM, using novel ester-linked fluorescent substrates, we rationalized substitutions to create an entirely ester-specific version (5000-fold preference), TssM*. We then asked whether TssM* would be a useful tool to aid the discovery of novel ubiquitin esters. In cell lysates, we identified several PARPs that were modified with both mono-ADP-ribose (MAR) and ubiquitin. We then used TssM* to show that PARPs contain two different ubiquitylated species: 1) canonically linked ubiquitin on PARP Lys residues; and 2) ester-linked ubiquitin conjugated directly to the MAR modification. This incredible finding was only made possible using TssM*, as the experimental standard treatment of NaOH or NH2OH would have completely removed the protein-conjugated MAR, masking the dual MAR-ubiquitin mark. We termed this dual modification MARUbylation and have since shown that other proteins recognize and further modify this signal. TssM* is a cornerstone for the study of non-canonical ubiquitylation and will rapidly accelerate the number and type of ubiquitin esters being studied, thereby adding a new level of complexity to the biological processes regulated by ubiquitylation.

#3 - The Salmonella Scaffold Protein SteE Rewires a Kinase Signaling Network to Evade Immune Detection

Mr. Hayden Henry, University of Washington
Mr. Phat Thien Luong, University of Washington

Ms. Avanti Bhandari, University of Washington
Prof. Rachel Klevit, University of Washington
Prof. Jesse Zalatan, University of Washington

The pathogen Salmonella typhimirium uses the secreted effector protein SteE to rewire host kinase signaling and prevent immune recognition. SteE is a scaffold protein that hijacks the host kinase GSK3, a canonical Ser/Thr kinase, to phosphorylate the nonnative substrate STAT3 on a tyrosine residue. We hypothesized that SteE promotes this unusual specificity switch through either induced proximity via tethering STAT3 or by reorganizing the GSK3 active site. Using quantitative kinetics, we found that SteE binding without substrate tethering is sufficient to rewire GSK3 specificity for preferential tyrosine phosphorylation. Unexpectedly, we also found that SteE dramatically decreases GSK3 activity for Ser/Thr substrates. These results suggest that SteE allosterically rewires GSK3 specificity to drive tyrosine phosphorylation. We next sought to understand if SteE alters GSK3 upstream regulation. SteE interacts with the same region in GSK3 as the AXIN scaffold and several other regulatory proteins. We previously demonstrated that AXIN protects GSK3 from upstream regulation by PKA. Using biochemical assays, we found that SteE also protects GSK3 from phosphorylation by PKA. Finally, we evaluated if SteE competes with other scaffold proteins for a limited GSK3 pool. Through in-vivo and in-vitro competition assays, we found that SteE forms a complex with active GSK3 that directly competes with the AXIN scaffold for GSK3. Together, these data indicate that SteE forms a stable and insulated signaling network with GSK3 by silencing competing reactions and protecting from upstream regulation.

#4 - Cysteine-activated Hydrogen Selenide (H2Se) Delivery from Isoselenocyanates

Mr. Reis Dorit, University of Oregon
Mr. Keyan Li, University of Oregon
Mr. Christopher A. Steven, University of Oregon
Dr. Arman G. Garcia, University of Oregon
Dr. Turner D. Newton, University of Oregon
Dr. Kaylin G. Fosnacht, University of Oregon
Prof. Michael D. Pluth, University of Oregon

Hydrogen selenide (H2Se) is an emerging biological signaling molecule and a central intermediate in selenium metabolism, analogous in reactivity and significance to small-molecule messengers such as NO, CO, and H2S. Despite its growing biological importance, few chemical tools exist for controlled H2Se delivery. Here, we report the development and characterization of tunable, cysteine-activated aryl isoselenocyanate (ISeC-R) H2Se donors. A series of electronically distinct ISeC-R derivatives were synthesized, and their H2Se release was monitored using a selective near-infrared H2Se-responsive fluorescent probe. H2Se release in ISeC-R donors were highly sensitive to substituent electronics, and the ISeC donor exhibited high selectivity for cysteine, enabling direct visualization of H2Se generation in live cells through fluorescence imaging. Overall, this work establishes a new class of tunable, cysteine-activated H2Se donors and provides a foundation for our understanding of selenium and targeted H2Se delivery in biological environments.

#5 - Mapping Structure-Function Relationships in Chimeric DcuS/EnvZ Histidine Kinases via Deep Mutational Scanning

Dr. Andrew Holston, University of Oregon
Samuel Hinton, University of Oregon
David Curtis, University of Oregon
Dr. Karl Romanowicz, University of Oregon
Dr. Calin Plesa, University of Oregon

A bacterium's ability to detect and respond to environmental changes is a prerequisite for survival. Bacteria use a wide range of two-component systems comprised of a sensor histidine kinase (SHK) and cognate response regulator to interact with their environment. SHKs detect a broad scope of ligands and stimuli, such as osmolarity, pH, organic compounds, and more. Following activation, the SHK phosphorylates its RR, which in turn influences gene expression to mediate cell response. Of the ~18 million putatively identified SHKs, only a few hundred are characterized. To bridge this gap, we take advantage of the high modularity of SHK protein structure to engineer chimeric HKs. We fused the sensory domain of the SHK DcuS with the transmember and cytosolic domains of EnvZ. The chimera communicates with an

orthogonal RR, which mediates transcription of GFP as a reporter. To find chimeras with novel functions, we constructed a 1.5 million CFU/mL mutant DcuS/EnvZ library. Here we screen the original DcuS/EnvZ chimera and mutant library against the known DcuS ligand and aspartate, which DcuS has no affinity for. Using a fluorescence-activated cell sorting (FACS) approach, we isolated twelve subpopulations of differentially activated chimeras under the three ligand conditions. By measuring the log-fold change in fluorescence relative to the no ligand control and leveraging deep mutational scanning (DMS), our study has identified mutant chimeras with altered functionality and shifted ligand specificity. This study represents one of the first to apply DMS to the SHK protein family, and our goals are to investigate the structure-function relationship of the DcuS sensory domain in a chimeric context to improve SHK chimera design principles. Our future studies aim to probe many SHK chimeras with varying sensory domains against a large ligand panel to characterize SHKs at scale.

6 - A new drug-dye conjugate, Adagrasib-OF650, allows for selective labeling of G12C PDAC tumors for fluorescence-guided surgery

Dr. Frederik Brøndsted, Oregon Health & Science University
Dr. Gauri Malankar, Oregon Health & Science University
Dr. Gourav Kumar, Oregon Health & Science University
Dr. Ge Huang, Oregon Health & Science University
Dr. Kai Tao, Oregon Health & Science University
Mr. Kyle Milnes, Oregon Health & Science University
Prof. Summer L. Gibbs, Oregon Health & Science University
Prof. Lei G. Wang, Oregon Health & Science University

Cancer is a leading cause of death in modern society, however, improvements in targeted therapies along with surgical interventions can dramatically improve patient survival. Kirsten rat sarcoma viral oncogene homolog (KRAS) is the most frequently mutated oncogene, and as such presents as an attractive drug target. Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer mortality and continues to increase in both incidence and fatality, thus improved therapy is urgently needed. Mutation rates of the KRAS oncogene are >90% in PDAC, where small molecule inhibitors such as Sotorasib and Adagrasib have been developed as potential treatments for KRASG12C mutant cancers. KRAS was previously thought to be undruggable, however the gain of a nucleophile through the glycine to cysteine mutation at codon 12 allowed the development of covalent KRAS targeted therapeutics. Herein, we describe a drug-dye conjugate utilizing the KRAS covalent inhibitor, Adagrasib as the molecular targeting agent, allowing specific labeling of KRASG12C. This novel probe could act as a companion imaging probe with applications for fluorescence guided surgery (FGS). Computational modeling was used to investigate the effects of appending our zwitterionic, environmentally inert fluorophore, OregonFluor650 (OF650) to Adagrasib. Adagrasib-OF650 (Ada-OF650) demonstrated similar docking scores to the unlabeled parent drug, Adagrasib. The selectivity of Adagrasib was retained as Ada-OF650 was able to distinguish between wildtype (WT), G12C, and G12D KRAS mutants both in vitro in purified proteins and in cell culture. The selectivity of Ada-OF650 was also demonstrated in vivo using orthotopic PDAC murine models. Specific uptake and labeling was demonstrated in KRASG12C mutation positive tumors compared to both KRASG12D mutation positive tumors and healthy pancreas tissue. The specificity of KRASG12C mutation positive tumor cell uptake and labeling were confirmed through fluorescence microscopy and histopathology of the resected PDAC tumor tissues. The development of Ada-OF650 highlights how creative linker chemistry can be used to take advantage of the tumor-specific targeting of approved small molecule therapeutics to create companion imaging tools for FGS approaches.

#7 - Development of a next-generation GCE tool for stable phosphorylation in mammalian cells

Dr. Jenna Beyer, Oregon State University
Moriah Mathis, Oregon State University
Dr. Ryan Mehl, Oregon State University
Dr. Rick Cooley, Oregon State University

Understanding how individual phosphorylation events regulate protein function in living cells remains a major challenge, as current methods cannot precisely install and preserve defined

phospho-marks. Serine phosphorylation is a widespread modification to proteins, yet it is highly dynamic and readily removed by cellular phosphatases, which limits mechanistic studies in cells. To overcome this barrier, we are developing a next-generation genetic code expansion (GCE) system to site-specifically encode a phosphatase-resistant phosphoserine mimic, non-hydrolyzable phosphoserine (nhpSer), in mammalian cells. To do this, we are (1) engineering more active aminoacyl-tRNA synthetases for pSer/nhpSer, (2) tuning cellular expression of GCE components, and (3) implementing a S. rubellomurinus biosynthetic pathway, which was previously used by our group in E. coli, to produce intracellular nhpSer in mammalian cells. Together, these efforts aim to establish a robust, user-friendly platform for direct encoding of stable phospho-marks in live mammalian cells. This system will be poised to precisely study signaling in live cells, such as the dissection of phosphorylation-dependent signaling networks and the creation of permanently phosphorylated proteins to mechanistically understand the relationship between phospho-signaling dysregulation and disease.

#8 - The effect of linker architecture on fluorescence properties of Riboglow-cobalamin probes to image RNA.

Prof. Aaron Van Dyke, Fairfield University
Mr. Luke Shafik, Georgetown University
Ms. Jenna Hanson, Fairfield University
Mr. Sebastian Lis, Fairfield University
Ms. Kiera Cunningham, Fairfield University
Ms. Brooke Tatarian, Fairfield University
Ms. Giulia Chitu, Georgetown University
Prof. Esther Braselmann, Georgetown University

Cobalamin (vitamin B12) is an organometallic, water-soluble vitamin. The chemoselective functionalization of cobalamin allows its targeted incorporation into an array of biochemical probes. Cobalamin is a key module of Riboglow, a fluorescent probe used to interrogate the location and dynamics of RNA in living cells. Cobalamin works in concert with two other modules: a fluorophore and a linker. These three modules together give rise to unique biophysical properties in the presence of RNA when studied by Fluorescence Lifetime Imaging Microscopy (FLIM). To systematically advance the Riboglow system, we must understand the factors governing interactions between modules. To achieve this goal, we disclose a revised synthesis that more convergently assembles the Riboglow probe. As a result, we present how the linker architecture alters the fluorescence intensity and fluorescence lifetime. We compare these second-generation probes to earlier Riboglow probes. The structure-property data gained from these studies informs the rational design of Riboglow probes for imaging RNA in living systems.

#9 - Deciphering the role of translation initiation factors using targeted protein degradation

Ms. Joy Chen, Fred Hutchinson Cancer Center
Dr. Annabel Olson, Fred Hutchinson Cancer Center
Ms. Christina Kuismi, Fred Hutchinson Cancer Center
Dr. Brandon Czowski, Fred Hutchinson Cancer Center
Dr. James Yelland, Fred Hutchinson Cancer Center
Dr. Hong Cao, University of Pittsburgh _x000D_
Mr. Andrew Weeden, University of Pittsburgh
Prof. Hua Zhang, University of Pittsburgh
Prof. Arvind Rasi Subramaniam, Fred Hutchinson Cancer Center
Prof. Andrew Hsieh, Fred Hutchinson Cancer Center
Prof. Behnam Nabet, Fred Hutchinson Cancer Center

Translation initiation factors coordinate the rate-limiting initiation step of protein synthesis, a tightly controlled process that is commonly dysregulated in tumorigenesis. While small-molecule inhibitors targeting translation initiation factors have shown anti-cancer effects, a lack of specific chemical tools has made it difficult to understand the molecular mechanisms behind how essential initiation factors control cell growth and survival. In this study, we aimed to rapidly and selectively degrade the eukaryotic translation initiation factor 1A X-linked (eIF1A), the only translation initiation factor recurrently mutated across multiple cancers. We hypothesized that degradation of eIF1A would rapidly disrupt overall protein synthesis and decrease cancer cell viability. To degrade eIF1A, we employed our targeted protein degradation technology platform known as the degradation tag (dTAG) system. In this approach, a heterobifunctional dTAG molecule binds to a universal protein tag (FKBP12F36V) fused to eIF1A, bringing it in proximity with an E3 ubiquitin ligase to induce rapid protein degradation.

We generated three isogenic pancreatic cancer cell lines by knocking out EIF1AX using CRISPR/Cas9 and complementing with transgene expression of EIF1AX-FKBP12F36V. Notably, we achieved rapid and dose-dependent loss of eIF1A-FKBP12F36V upon recruitment of the E3 ubiquitin ligase VHL, in as little as one hour. eIF1A degradation led to a striking reduction in nascent translation within one hour of dTAG molecule treatment, revealing a heightened sensitivity to the absence of eIF1A. Next, we used quantitative proteomics to understand the time-dependent proteome-wide changes that occur upon eIF1A loss. This assessment revealed the specificity of eIF1A degradation at acute time-points, and downregulation of several critical transcriptional regulators including SOX4 within four hours. We also observed the activation of the ribotoxic stress response within eight hours of eIF1A degradation, as evidenced by increases in JUN and ATF3 protein levels. The ribotoxic stress response occurs when translation elongation is impaired and stimulates stress-activating proteins that affect cell fate and proliferation. Accordingly, the degradation of eIF1A significantly reduced cell viability in both 2D-monolayer and 3D-spheroid conditions, highlighting the inability of cancer cells to overcome this severe block in protein synthesis. Our application of the dTAG system to study eIF1A now allows for granular dissection of the ribotoxic stress response and the biological consequences of targeted degradation of eIF1A in vitro and in vivo. Together, our work highlights the application of targeted degradation approaches to interrogate translation initiation factors with speed and precision.

#10 - Peptide Inhibitors of STING Oligomerization

Dr. Xujun Cao, Arc Institute
Mr. Eu-Tchen Ang, University of Tokyo
Dr. Rachel E. Mardjuki, Arc Institute
Dr. Daniel Fernandez, Stanford University
Dr. Christine Wolf, Dresden University of Technology
Prof. Min Ae Lee-Kirsch, Dresden University of Technology
Prof. Hiroaki Suga, University of Tokyo
Prof. Lingyin Li, Stanford University and Arc Institute

The innate immune pathway STING is often erroneously activated during autoimmune syndromes and interferonopathies, including ageing-related inflammation. An ongoing effort to identify broadly effective STING inhibitors is further complicated by the diversity of common human STING alleles. We used the RaPID platform to identify peptide binders of STING at nanomolar affinity. Crystallography showed that peptides leveraged previously untargeted binding sites. Of these, a high-affinity peptide, ET6, targets STING oligomerization, the most fundamental molecular aspect of STING activation. Structural analysis reveals that ET6 competitively disrupts the rigid alignment between STING's cytosolic and transmembrane domains, which is normally enforced by STING's N-terminal tail. ET6 inhibits STING activation by cGAMP in primary PBMCs from donors of all major STING haplotypes. ET6 also inhibits constitutively active STING mutants clinically associated with SAVI, substantiating a promising lead inhibitor candidate for inflammatory conditions.

#11 - Development and Application of a HTS-Based Recorder of Ras Signaling

A. Felicia Adebanjo, University of Washington
Dr. Dustin Maly, University of Washington

Development and Application of a HTS-Based Recorder of Ras Signaling Ras is a small GTPase whose activity is regulated by many proteins that switch it between an "on" state (GTP-bound) and an "off" state (GDP-bound). Once activated, Ras signals to several key effectors involved in critical cellular pathways. Despite its central role in signaling, there is currently no method that can efficiently and accurately measure Ras activation at scale. To address this challenge, we developed a Ras activity reporter compatible with high-throughput screening (HTS). In this system, Ras activation is recorded through a biotinylation event that depends on the interaction specific to the active state. An RNA molecule linked to Ras serves as the HTS readout of its activity. We validated the reporter's ability to distinguish between constitutively active and native Ras states and demonstrated a time-dependent response, confirming that it can capture even transient Ras activation. The high throughput nature of this technology enables the study of large variant

libraries of upstream Ras regulators, offering new insights into parts of the signaling network that are often hidden when using further downstream readouts. Together, this system establishes a scalable and quantitative recorder of Ras activation. Furthermore, it establishes a general framework for developing high-throughput screening (HTS)–based recorders for other ~150 GTPases in the Ras super family. By combining these GTPase activity recorders with protein variant libraries and systematic studies of cellular signaling, we aim to understand how single mutations in large, multidomain proteins can reroute signals and change cell behavior.

#12 - Cyclic immunofluorescence (cyCIF) using antibodies with cleavable fluorophores to elucidate prostate adenocarcinoma progression

Ms. Jocelyn A. Jones, Oregon Health & Science University
Dr. Frederick Brondsted, Oregon Health & Science University
Ms. Cam Nguyen, Oregon Health & Science University
Dr. Cody Rounds, Oregon Health & Science University
Dr. Lei G. Wang, Oregon Health & Science University, Knight Cancer Institute
Dr. Ryan P. Kopp, Oregon Health & Science University, Knight Cancer Institute
Dr. Melissa H. Wong, Oregon Health & Science University, Knight Cancer Institute
Dr. Summer L. Gibbs, Oregon Health & Science University, Knight Cancer Institute

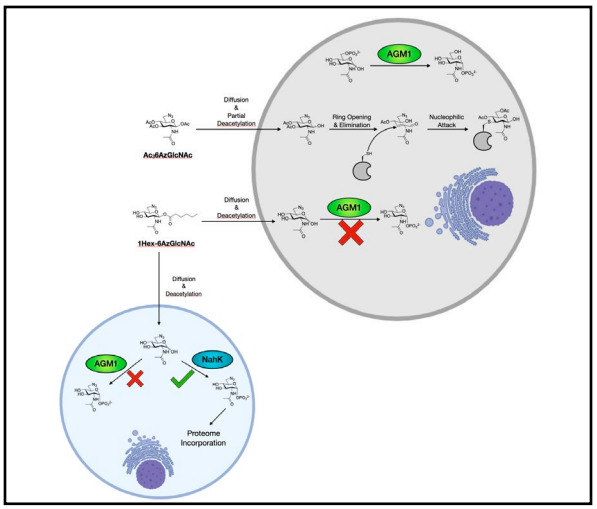
Highly multiplexed imaging technologies continue to advance, improving our understanding of the importance of disease spatial biology including cancer initiation, progression and metastasis. Cyclic immunofluorescence (cyCIF) has been instrumental in generating vast datasets that can be assessed from the single cell level to understand contextual cues at the tumor and organ-wide level. The quantitative information generated from a single formalin-fixed paraffin embedded (FFPE) tissue sample is substantial but represents only a snapshot of disease development. When paired with patient matched lymph nodes and blood samples, a full picture of disease progress and metastatic potential is possible. Herein, using patient matched tumor tissue, lymph nodes and blood, we leverage our existing antibody oligonucleotide (Ab-oligo) cyCIF platform. We expanded the use of removable fluorophores to incorporate direct and indirect antibody labeling into our cyCIF platform, permitting us to expand our biomarker library while maintaining gentle signal removal between rounds. This was particularly helpful for low abundance antigens whose fluorescence signal to background often made detection difficult in the crowded tissue environment. A particular biomarker gaining interest recently is tumor-immune hybrid fusion cells. These cells are characterized by the co-expression of CD45, a macrophage marker, and at least one epithelial marker of tumor origin (e.g., pan-cytokeratin, E-Cadherin or Epcam). In tissue, these hybrid cells are often located near vessels where they are thought to promote angiogenesis and cell motility. In blood, these circulating hybrid cells (CHCs), which exist at an order of magnitude higher numbers than conventional circulating tumor cells (CTCs), are a biomarker for disease progression and metastases. We leveraged our cyCIF platform to quantify hybrid cell subpopulations in matched patient blood and tissue, allowing downstream phenotypic analysis to predict tumor prognosis.

#13 - Molecular Analysis of Ganaspis xanthopoda Venom Reveals Targeted Inhibition of JAK-STAT-Mediated Immune Signaling in Drosophila melanogaster Hemocytes

Mr. Riitvek Baddireddi, Oregon State University
Prof. Nathan Mortimer, Oregon State University

The JAK-STAT signaling pathway is a highly conserved cascade essential for regulating immune responses, cell proliferation, and hematopoiesis. Dysregulation of this pathway contributes to numerous human diseases, including autoimmune disorders, immunodeficiencies, and cancers such as leukemia and lymphoma. Despite its importance, the mechanisms underlying in vivo modulation of JAK-STAT signaling remain incompletely understood. Drosophila melanogaster provides a powerful genetic model for studying this pathway, as its core components and regulatory architecture are evolutionarily conserved. This study aims to characterize the components of Ganaspis xanthopoda venom that suppress JAK-STAT signaling in D. melanogaster. The mutant line hop^Tum-l Stat92E-GFP serves as a model

for monitoring JAK-STAT signaling via GFP expression in immune cells. Second-instar larvae were parasitized by the wasp, and cytometric analysis of bled hemocytes revealed an average 8.7% reduction in relative fluorescence units (RFU) compared to controls ($p = 2.25 \times 10\text{-}3$). Venom was extracted and purified, then applied directly to bled immune cells, resulting in a similar RFU decrease of 11.78% ($p = 4.18 \times 10\text{-}11$). To isolate active components, venom was fractionated using a 10–50% sucrose gradient and dialyzed to replace the sucrose solution with 1× PBS, restoring physiological conditions for subsequent assays. When these fractions were tested on bled hemocytes, only fraction 3 significantly suppressed GFP expression, reducing RFU by 3.02% ($p = 2.10 \times 10\text{-}2$). Following sonication, fraction 3 lost inhibitory activity (RFU decreased by 2.08%, $p = 0.205$),



suggesting that the active protein is water-soluble and relies on vesicular transport to enter target cells. Heat denaturation did not eliminate inhibitory activity (RFU decreased by 2.30%, $p = 2.16 \times 10\text{-}2$), indicating that venom vesicles protect the protein from thermal degradation. Fractions 1–5 were analyzed via Tunable Resistive Pulse Sensing (TRPS) to determine vesicle size distributions. Fraction 1 could not be analyzed due to low vesicle content; fractions 2, 4, and 5 displayed heterogeneous vesicle populations, while fraction 3 exhibited a predominant vesicle population with a mean diameter of 120 nm. SDS-PAGE analysis of fractions 1–5 revealed a prominent protein band in fraction 3 at approximately 50 kDa, suggesting a potential candidate responsible for the observed JAK-STAT inhibitory activity. RNA was isolated from Ganaspis xanthopoda, and 1% agarose electrophoresis showed intact 18S (~1.9 kb) and 28S (~4.7 kb) rRNA bands, indicating high RNA integrity suitable for transcriptomic analysis. The 28S rRNA exhibited a species-specific 'hidden break,' a common feature in insects that can cause the band to appear faint or split under denaturing conditions, reflecting a structural feature rather than degradation. This RNA is being used to generate a transcriptome, and fraction 3 has been submitted for mass spectrometry analysis. These datasets are being integrated to identify candidate venom proteins that inhibit JAK-STAT signaling. This work provides a foundation for identifying novel immunomodulatory factors and offers insight into the conserved mechanisms of JAK-STAT regulation. The findings have implications for developing targeted therapies using venom-derived proteins to modulate JAK-STAT signaling in humans, which could aid in understanding and treating diseases such as rheumatoid arthritis, lupus, psoriasis, myeloproliferative neoplasms, certain lymphomas, and related pathologies.

#14 - A Cell-Specific O-GlcNAc Metabolic Chemical Reporter

Mr. Geoffrey Lang, University of Southern California_x000D_
Miss. Muerielle Morales, University of Southern California_x000D_
Miss. Jiaxin Cui, University of Southern California_x000D_
Prof. Matthew Robert Pratt, University of Southern California

Figure 1. (A) AGM1 isomerizes its native substrate GlcNAc-6-phosphate to GlcNAc-1-phosphate. (B) $Ac_3$6AzGlcNAc can be partially deacetylated at the 1-position leading to the formation of an α-β-unsaturated carbonyl. Cysteines on cytosolic proteins can react with this Michael-acceptor causing non-specific labeling. (C) 1Hex-6AzGlcNAc is not metabolized by AGM1, but NahK phosphorylates the 1-position leading to incorporation of the probe into the cell's proteome.

A hallmark of cancer metabolism is the misregulation of protein post-translational glycomodifications. O-GlcNAcylation is upregulated in cancer and linked to metastasis,

chemoresistance, and poor prognoses. Researchers have discovered diagnostic biomarkers and therapeutic targets, such as HMGB1, are O-GlcNAc modified in cancer. We sought to leverage the upregulation of O-GlcNAc modification in malignancies to develop a small-molecule probe and biological system to identify biomarkers in cancer versus healthy tissue.

Metabolic chemical reporters (MCRs) are small-molecule probes for studying glycomodification by allowing researchers to visualize glycosylated proteins *in vivo* and enrich them to identify glycosylation sites. MCRs are typically analogs of carbohydrates with an azide or alkyne to link fluorophores or enrichment tags via click-chemistry. Most MCRs are not specific for a single class of glycosylation (e.g. O-GlcNAc, mucins, N-glycans), limiting the information on different modification processes. Several MCRs were reported to be specific for O-GlcNAcylation, such as $Ac_3$6AzGlcNAc. However, these first-generation MCRs are per-O-acetylated for cell permeability but this feature also causes non-specific labeling of cysteines, which introduces false positives into the readout (Fig 1B).

We designed a new O-GlcNAc specific MCR, 1Hex-6AzGlcNAc which has a hexanoate chain for cell permeability and an azide for click-chemistry. Previous studies showed that $Ac_3$6AzGlcNAc is a poor substrate for a key enzyme in MCR metabolism, AGM1. AGM1's native function is isomerizing the phosphate from the 6-OH to the 1-OH of N-acetyl glucosamine (GlcNAc) (Fig 1A). However, neither 1Hex-6AzGlcNAc nor $Ac_3$6AzGlcNAc contain a 6-OH to facilitate the isomerization of a phosphate. We overcame this bottleneck by engineering K-562 cells that express the bacterial enzyme, N-acetylhexoseamine kinase (NahK), which directly phosphorylates the 1-OH of GlcNAc. This process bypasses AGM1 and allows for subsequent incorporation into the proteome (Fig 1C).

When K-562 (NahK) cells were treated with 1Hex-6AzGlcNAc, there was robust labeling of the proteome. This result was repeated in HEK293T cells expressing NahK, showing broader application across multiple cancer cell lines. Follow-up biochemical characterization, including deep proteomics, indicates that 1Hex-6AzGlcNAc specifically labels O-GlcNAc modified proteins without falsely labeling aberrant cysteine residues.

We also evaluated the cell-specificity of 1Hex-6AzGlcNAc for NahK-positive cells over wild-type cells. K-562 (NahK) and K-562 (mCherry) cells were co-cultured and treated 1Hex-6AzGlcNAc. Flow cytometry analysis showed that only K-562 cells expressing NahK incorporated 1Hex-6AzGlcNAc into their proteome, indicating our MCR is cell-specific. A similar experiment was performed to selectively enrich a known O-GlcNAc modified target, HMGB1. HEK293T (NahK/HA-HMGB1) and HEK293T (FLAG-HMGB1) co-cultured and treated with 1Hex-6AzGlcNAc. A streptavidin pull-down assay revealed that HA-HMGB1 was selectively enriched over FLAG-HMGB1 confirming the cell-specific enrichment of an O-GlcNAc modified protein.

Since many secreted proteins in cancer are O-GlcNAc modified, our cell-specific O-GlcNAc MCR, 1Hex-6AzGlcNAc, shows promise for identifying diagnostic biomarkers, because this probe can differentiate secreted proteins from malignancies versus healthy tissue.

#15 - 5-thio carbamate analogs of N-acetylglucosamine inhibit the hexosamine biosynthetic pathway through a novel mechanism

Ms. Rhianna Haynie-Cion, University of Southern California
Dr. Moira Morales, University of Southern California
Dr. Nichole Pedowitz, University of Southern California
Dr. Matthew Pratt, University of Southern California
Dr. Jennifer Kohler, University of Texas, Southwestern
Dr. Costas Lyssiotis, University of Michigan
Dr. Dorothea Fielder, Humboldt University of Berlin
Dr. Ben Schumann, The Francis Crick Institute

N-acetylglucosamine (GlcNAc) is a dynamic post-translational modification found on intracellular proteins, functioning as a metabolic sensor and playing essential roles in mammalian development and cell survival. This vital role makes it difficult to study using traditional genetic approaches, prompting the development of chemical analogs to probe and inhibit the hexosamine biosynthetic pathway. One such analog, 5-thio-GlcNAc (5SGlcNAc), is metabolized through the GlcNAc salvage

pathway into UDP-5SGlcNAc - a well-characterized inhibitor of O-GlcNAc transferase (OGT) - and also induces feedback inhibition of GFAT, an upstream enzyme. To enable cell-specific inhibition of OGT, as full elimination of O-GlcNAc is embryonic lethal, we employed a "bump-hole" strategy targeting the enzyme AGX1, which cannot process GlcNAc analogs with bulky N-acetyl substituents unless mutated (F383G/A). We synthesized a panel of such 5SGlcNAc analogs and tested them in wild-type cells. Unexpectedly, all analogs reduced O-GlcNAcylation even without the AGX1 mutation. Focusing on one analog, 5SGlcNTroc, we used cellular metabolomics to show that cells do not produce UDP-5SGlcNTroc. Unlike 5SGlcNAc, it also does not reduce UDP-GlcNAc levels, suggesting a distinct mechanism of O-GlcNAc reduction. Through a combination of in cellulo assays and in vitro enzyme screening with chemically synthesized 5SGlcNTroc metabolic intermediates, we believe that 5SGlcNTroc likely inhibits AGM1, a key enzyme in both the hexosamine biosynthetic and GlcNAc salvage pathways. Pancreatic cancer cells rely on elevated O-GlcNAc levels for proliferation. Although upstream pathway inhibitors have been explored, cancer cells can restore GlcNAc levels by salvaging monomers from hyaluronic acid breakdown. By targeting AGM1, a convergence point of both pathways, 5SGlcNTroc offers a promising strategy for therapeutic intervention.

#16 - Proteomics-by-Sequencing: Applying LABEL-seq for High-Throughput Functional Profiling of the Human Proteome

Haden Wikar, University of Washington
Madeline Walsh, University of Washington
Felix Tsai, University of Washington
Dr. Jessica Simon, University of Washington
Dr. Dustin Maly, University of Washington

Recent advances in quantitative mass spectrometry over the past decade have greatly increased both the depth and breadth of protein measurements, enabling proteomic studies that offer insight into complex biological systems. Despite these gains, mass spectrometric methods remain limited in their throughput and ability to comprehensively profile diverse functional properties of proteins. An attractive alternative to these traditional approaches lies in DNA sequencing-based technologies, which combine high-throughput analysis with a sensitive and accurate readout, offering the potential to analyze a wide range of protein properties. High-throughput sequencing (HTS)-based methods rely on nucleic acid barcodes, which can be coupled to protein identity and biochemical state. However, there are currently no HTS-based methods that enable proteome-scale study and quantification of protein biochemistry. Here, we present recent work to develop a proteomics-by-sequencing approach, which leverages an HTS-based proteomic platform called LABEL-seq. LABEL-seq relies on the co-expression of a protein-of-interest and a cognate RNA barcode that encodes the protein ID. Importantly, these two components self-assemble and remain stably associated through lysis and affinity enrichment, allowing for the quantification of 1000s of proteins with HTS in a single pooled experiment. We have generated proof-of-concept LABEL-seq libraries with hundreds of genes using cloning strategies that are compatible with commercially available ORF libraries, allowing these libraries to be readily expanded to include the entire proteome. We also developed an analysis pipeline for full-plasmid sequencing data that maps each RNA barcode to its corresponding protein. The initial functional protein properties that are being profiled with the proteome-scale libraries include interaction, activity, and druggability, providing an expandable framework for further proteomic analysis. Collectively, this approach overcomes major limitations of existing proteomics methods, offering a scalable and functional window into previously inaccessible regions of the human proteome.

#17 - GPCR antagonism via antibody-mediated rewiring of receptor trafficking and degradation

Kaitlin Rhee*, Dana-Farber Cancer Institute, Harvard Medical School
Lawrence Shue*, Dana-Farber Cancer Institute, Harvard Medical School
Dr. James Osei-Owusu, Harvard Medical School
Dingjingyu Zhou, Dana-Farber Cancer Institute, Harvard Medical School
Dr. Aoxing Cheng, Dana-Farber Cancer Institute, Harvard Medical School
Dr. Apoorva Baluapuri, Harvard Medical School, Eli & Edythe L. Broad Institute

Dr. Edward P. Harvey, Harvard Medical School
Prof. Karen Adelman, Harvard Medical School, Eli & Edythe L. Broad Institute
Prof. Meredith A Skiba, Harvard Medical School, University of Michigan School of Medicine
Prof. Andrew C. Kruse, Harvard Medical School
Prof. Xin Zhou, Dana-Farber Cancer Institute, Harvard Medical School

GPCRs are the largest superfamily of cell surface receptors, with over 800 members known to exist in humans. GPCRs play key roles in human physiology and diseases, including cancer, autoimmunity, neuropsychiatric disorders and cardiovascular diseases. Conventional antagonism is challenged by constitutive signaling, high ligand affinity/concentration, and highly conserved orthosteric binding sites. We have developed a bispecific antibody-based approach for GPCR antagonism via induced proximity to transferrin receptor 1 (TfR1), co-internalization and degradation in lysosomes. This approach harnesses the rapid constitutive endocytosis of TfR1, a broadly expressed cell surface receptor that mediates iron import. We demonstrated the ability of GPCR-TfR1 targeting chimeras (GTACs) to induce potent TfR1- and lysosome-dependent depletion and signaling inhibition across a range of targets, including AT1R (a peptide hormone receptor critical for blood pressure regulation), RXFP1 (a peptide hormone receptor implicated in ovarian cancer metastasis and cisplatin resistance), and CCR6 (a chemokine receptor involved in T cell-mediated autoimmunity). In a case-study of AT1R, a prototypical GPCR, AT1R GTACs routed AT1R to endosomes and lysosomes to achieve sustained degradation and inhibition, distinct from native ligand-induced transient internalization and recycling patterns. RXFP1 GTACs suppressed downstream metastatic gene expression in ovarian cancer cells, while CCR6 GTACs inhibited primary T cell migration. We showed the GTAC platform is compatible with a range of antibody scaffolds and binder formats, such as anti-GPCR Fab/VHH and anti-TfR1 scFv/VHH. Furthermore, anti-FLAG GTACs provide a generalizable tool for rapid on-demand downregulation and antagonism of FLAG-tagged GPCRs without the use of a target-specific binder. GTACs represent a new modality for pharmacological modulation of GPCRs, where GPCR levels and activities can be precisely controlled by induced proximity to TfR1. In addition to their therapeutic potential, these molecules may serve as tools to dissect the spatial organization of GPCR signaling.

#18 - Reliable Dual Encoding Systems for Genetic Encoding of Noncanonical Amino Acids

Mr. Nathan Palazzolo, Oregon State University
Ms. Lanelle Connolly, Oregon State University
Prof. Ryan Mehl, Oregon State University
Dr. Richard Cooley, Oregon State University

Genetic code expansion (GCE) enables site-specific installation of noncanonical amino acids (ncAAs) to build proteins with post-translational modifications, biophysical probes and conjugation handles, among many others. While encoding a single ncAA has become relatively common, constructing multifunctional proteins that require dual ncAA incorporation remains challenging due to orthogonal pair cross-reactivity, limited codon capacity, and low expression yields that often cap performance and limit applications. To overcome these constraints and to realize the unique advantages of dual encoding, we have developed modular dual-ncAA platforms in E. coli that combine evolved tRNA/tRNA-synthetase pairs from Methanomethylophilus alvus, Methanocaldococcus jannaschii, and Methanococcus maripaludis. These systems support function-specific ncAA combinations, including (i) non-hydrolyzable phosphoserine (nhpSer) and tetrazine to generate stably phosphorylated proteins that can be site-selectively labeled with fluorophores and introduced into eukaryotic environments, and (ii) acridone and tetrazine to enable intramolecular FRET-based measurements of protein conformational changes. Rational plasmid architectures and expression condition optimization increased dual-encoded protein yields up to fourfold relative to prior reports, with product identity confirmed by mobility-shift assays and mass spectrometry. Together, these advances deliver practical dual-ncAA toolkits that can (a) report on phospho-proteoforms in complex environments, (b) provide fluorescent and bioorthogonal handles for real-time fluorescence-based studies, and (c) open new routes to site-specific conjugations relevant to therapeutic design. By making dual encoding reliable and application-oriented, our platforms

broaden the experimental landscape beyond what is accessible with single encoding of noncanonical amino acids.

#19 - PINBody: an inclusion body strategy for production of unmodified and phosphorylated intrinsically disordered proteins for mechanistic and structural studies

Mrs. Cat Hoang Vesely, Oregon State University
Dr. Stanislau Stanisheuski, Oregon State University
Edward Lien, University of Southern California
Patrick Reardon, Oregon State University
Peter J. Chung, University of Southern California
Ryan A. Mehl, Oregon State University
Richard B. Cooley, Oregon State University

Phosphorylated intrinsically disordered proteins (IDPs) such as Bcl2-associated agonist of cell death (BAD) and Tau play critical roles in apoptosis and neurodegeneration, yet their high flexibility and solvent exposure render them prone to degradation, aggregation, and dephosphorylation during recombinant expression, limiting biophysical and structural analysis. Here, we introduce the PINBody tag (PTM IDP Inclusion Body), a versatile inclusion body (IB)-targeting fusion that protects IDPs from degradation during expression in E. coli. When combined with genetic code expansion (GCE), PINBody enables site-specific incorporation of phosphoserine (pS) while preserving this labile modification from dephosphorylation. Unlike previous IB-tag systems that rely on harsh chemical cleavage incompatible with large, sequence-complex IDPs and labile PTMs, PINBody supports rapid, protease-mediated tag removal and recovery of the target phosphorylated IDP under mild conditions. We demonstrate the platform's general utility by producing milligram-scale quantities of mouse BAD phosphorylated at S136 and human 2N4R Tau phosphorylated at S404. Using PINBody, we also generated homogeneous ^{15}N-labeled Tau pS404 and performed 2D HSQC NMR. Phosphorylation at S404 induced local and distal chemical shift perturbations and primed efficient GSK3β phosphorylation at S400 and S396, yielding the triply phosphorylated Tau proteoform consistent with the proposed "zippering" mechanism. In contrast, GSK3β phosphorylation of unmodified Tau was incomplete without priming. Together, these results establish PINBody as a robust, generalizable platform for producing native and site-specifically modified IDPs, overcoming longstanding technical barriers and enabling mechanistic dissection of phosphorylation cascades in Tau and other disease-relevant proteins.

#20 - α-Synuclein sequences from long-lived animals display generally diminished aggregation compared to shorter-lived animals including humans

Gilbert Boadu Ampomah, University of Southern California
Dr. Eldon Ralph Hard, Albert Einstein College of Medicine
Dr. Matthew Robert Pratt, University of Southern California

Many neurodegenerative diseases are caused by the formation of misfolded protein aggregates in different regions of the brain. These aggregates are characterized by the stacking of protein monomers in β-sheets. They are thought to spread between neurons and have been shown to be directly toxic to neurons and other cells in culture. α-synuclein is the amyloid forming protein in Parkinson's disease and other synucleinopathies like multiple systems atrophy (MSA). Most cases of PD are sporadic with the onset of symptoms happening later in life (55-65 years). Some point mutations and gene duplication/triplication that cause early onset PD are still towards the end of the human lifespan. However, SNCA is an ancient gene that can be found in all vertebrates, some of which are very long lived and does not reach reproductive maturity until after 100 years. Hence, the development of PD could be detrimental to such species. Therefore, we hypothesized that long-lived organisms might harbor α-synuclein mutations that slow down aggregation compared to the human sequence. Here, we used recombinant protein expression and biophysical techniques to probe the aggregation propensity of α-synuclein from short-lived (mouse, chicken, frog) and long-lived (elephant, crocodile, shark, whale) species relative to the human variant. Proteins from long-lived species showed slower aggregation rates according to the Thioflavin T kinetic assay whereas that from the short-lived species, including human, aggregated between 8-72 hours. Proteinase K digestion, sedimentation,

circular dichroism, and transmission electron microscopy together suggest that aggregates formed by the long-lived species are morphologically distinct and unstable relative to human, except for the elephant variant which showed no formed aggregates. Altogether, our results suggests that there might be evolutionary pressure to slow down the aggregation and accumulation of α-synuclein in longer living organisms.

#21 - Optical Control of GPR55-Mediated Ca2+ Signaling in HEK293 Cells with Photopharmacology

Gabriella Calvi, Oregon Health & Science University_x000D_
Dr. James A. Frank, Oregon Health & Science University

The endocannabinoid system is ubiquitous throughout the human body, playing an essential role to maintain homeostasis. It regulates sleep, appetite, memory, and immune responses through cannabinoid receptors CB1 and CB2, as well as noncanonical receptors and ion channels. GPR55, an orphan G protein–coupled receptor (GPCR), is often regarded as a third cannabinoid receptor due to its widespread expression throughout the central nervous system and activation by cannabinoids such as (−)-trans-Δ9-tetrahydrocannabinol (THC). GPR55 has been implicated in pain signaling, inflammation, and glucose homeostasis. At the molecular level, GPR55 couples to Gαq proteins to activate RhoA GTPase and phospholipase C, leading to intracellular Ca2+release from the endoplasmic reticulum. Despite its physiological relevance, the mechanisms GPR55 uses to mediate its biological functions remain poorly understood, in part due to its low ligand selectivity and lipid nature of its ligands. This limitation makes it challenging to attribute observed effects specifically to this receptor among other cannabinoid receptors. Furthermore, there is a need for chemical tools that allow for increased spatial and temporal modulation of GPR55 activity. This research aims to address this limitation by developing a photoswitchable ligand capable of reversibly activating GPR55 with ultraviolet light. Our initial work focuses on establishing a method for evaluating ligand efficacy by optimizing a real-time Ca2+ imaging assay in live HEK293 cells. This assay will be used to quantify intracellular Ca2+ response following a drug addition. Once optimized, we will use this assay to guide and evaluate the design of a novel azobenzene-based photoswitchable ligand that achieves reversible control of GPR55. This optical tool will be used for selectively probing GPR55-mediated Ca2+ signaling in real time to evaluate cell networks within complex biological environments. Ultimately, GPR55 is an integral component of the endocannabinoid system and this work will provide new avenues to manipulate GPR55 with unprecedented spatiotemporal control.

#22 - Retromer complex protects the mu opioid receptor from opioid-induced downregulation

Mr. Hayden Adoff, Oregon Health & Science University
Ms. Aleksandra Dagunts, Oregon Health & Science University
Mr. Brandon Novy, Oregon Health & Science University
Ms. Monica de Maria, Oregon Health & Science University
Dr. Braden Lobingier, Oregon Health & Science University

A crucial step in homeostatic regulation of G-protein coupled receptor (GPCR) activity is agonist-induced downregulation of the GPCR via endocytosis and trafficking to the lysosome for degradation. This process can be opposed by sequence-dependent sorting in which cytoplasmic facing portions of receptors containing specific recycling motifs allow for binding of protein complexes that sort proteins to be removed from the maturing endolysosome and return them to the plasma membrane. The mu opioid receptor (MOR) is one clinically relevant GPCR, responsible for the effects of opioid drugs, which undergoes agonist induced downregulation with prolonged stimulation with high efficacy agonists, but can also partially recycle. MOR contains a novel bileucine motif (LENL) and can recycle via a previously unknown pathway. To identify determinants of MOR downregulation and/or recycling, we used a genome wide CRISPR interference screen and functional assay for agonist-induced downregulation. Our screen showed that the Retromer complex is a critical regulator of the

trafficking itinerary of MOR, with more agonist-induced degradation after Retromer knockdown. We confirmed that MOR recycles through the Retromer complex, and that the non-canonical bileucine motif is required for MOR to recycle via this pathway. Moving forward, we are taking a two-pronged approach to elucidate the mechanistic basis for the MOR-Retromer interaction, in cells with a KO-rescue strategy, and in vitro by purifying the Retromer complex.

#23 - De novo design of phospho-tyrosine peptide binders

Dr. Jason Z. Zhang, Department of Biochemistry, University of Washington
Dr. Magnus S. Bauer, Department of Biochemistry, University of Washington
Dr. Kejia Wu, Department of Biochemistry, University of Washington
Dr. Gyu Rie Lee, Department of Biochemistry, University of Washington, Seattle, WA, USA; Institute for Protein Design, University of Washington, Seattle, WA, USA; Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, South Korea
Dr. Brian Coventry, Department of Biochemistry, University of Washington, Seattle, WA, USA; Institute for Protein Design, University of Washington, Seattle, WA, USA
Prof. David Baker, Department of Biochemistry, University of Washington, Seattle, WA, USA; Institute for Protein Design, University of Washington, Seattle, WA, USA; Howard Hughes Medical Institute, University of Washington, Seattle, WA, USA

Key events in cellular signaling involve the phosphorylation of tyrosine residues, often on transmembrane receptor intracellular regions, and hence the detection and modulation of tyrosine phosphorylation is of great interest. Despite recent progress in de novo design of binding proteins, there are no current methods for designing binders that recognize phosphotyrosine and other protein covalent modifications in a broader sequence context, often within unstructured regions. Here we introduce RoseTTAFold Diffusion 2 for All-Atom Molecular Interfaces (RFD2-MI), a deep generative framework for design of binders for protein, ligand, and covalently modified protein and peptide targets. We demonstrate the power and versatility of this method by designing binders for critical phosphotyrosine sites on three distinct and clinically relevant targets: Cluster of Differentiation 3 (CD3ε), Epidermal Growth Factor Receptor (EGFR) and Insulin Receptor (INSR). Following sequence design with LigandMPNN and orthogonal complex prediction using AlphaFold 3, the resulting binders were experimentally validated to recognize their targets with both sequence and phosphorylation specificity. They bound phosphorylated targets with affinities as low as 500 nM, showed much reduced or negligible binding to non-phosphorylated targets or off targets, and exhibited structural agreement within 1 Å RMSD between predicted and crystal structures. RFD2-MI provides a generalizable all-atom diffusion framework for probing and modulating phosphorylation-dependent signaling, and more generally, for developing research tools and targeted therapeutics against PTM modified proteins.

#24 - DNA Pro-Retention Modulators of PARP1 Overcome Primary and Acquired Resistance to Clinical PARP Inhibitors

Jonathan Tullis
Kapil Upadhyaya
Michael Cohen

PARP1 inhibitors (PARP1i) improve outcomes in BRCA-deficient cancers, but broader use is limited by resistance, and the features required for efficacy remain unclear. Although efficacy is often attributed to “PARP trapping,” recent evidence has challenged this dogma. Mechanistically, PARP1i are more appropriately classified by their allosteric effects on PARP1–DNA affinity: type I (pro-retention), type II (neutral), and type III (pro-release). Using a diverse panel of compounds, including enantiomeric pairs that uncouple catalytic inhibition from allostery, we define how inhibitor type links to cellular and in vivo phenotypes. Several orthogonal methodologies revealed that PARP1 catalytic inhibition alone was sufficient for synthetic lethality in BRCA-deficient cells. In contrast, type I behavior was required for cytotoxicity in BRCA-reversion and BRCA-wildtype cancer cells and in vivo xenograft models. These results clarify the mechanistic basis for PARP1i efficacy and establish type I allosteric modulators as a promising strategy for overcoming clinical resistance to PARP1i.

#25 - Identification of Zika Virus Host Factors via Functionalized Sphingolipid Probes

Dr. Judah Evangelista, Oregon Health & Science University
Dr. Scotland Farley, Oregon Health & Science University
Prof. Fikadu Tafesse, Oregon Health & Science University
Prof. Carsten Schultz, Oregon Health & Science University

Zika virus and other flaviviruses induce major changes in lipid metabolism of infected cells, commonly upregulating ceramide and other members of the sphingolipid family. Sphingolipids play many important signaling roles controlling diverse cellular fates such as differentiation, proliferation, and cell death, but their specific roles in viral infection are largely unknown. Characterizing the sphingolipid interactome - the set of proteins that directly bind to sphingolipids - is a promising strategy to identify new viral host factors and clarify the mechanistic roles of these under-studied lipids. In this study, we used functionalized, crosslinkable analogs of sphingolipid bases and fatty acids in pulldown proteomics experiments to define lipid interactomes in healthy vs. Zika-infected liver epithelial cells. We observed major differences between the two sets and identified hundreds of proteins specifically enriched in sphingolipid interactomes during infection. Sphingolipid interactomes encompassed both well-characterized flavivirus host factors and proteins with no previous link to infection that may represent novel drug targets. To classify novel candidates as pro- or anti-viral factors, we conducted a pooled CRISPR knockout screen for Zika virus resistance in Huh7 cells targeting the most highly enriched and specific sphingolipid-interacting proteins. Top hits from this screen included members of the nuclear ribosome assembly complex and the membrane-trafficking Rab family. We further confirmed hits using cellular knockout/rescue studies assessing various outcomes of Zika virus infection, as well as high-resolution fluorescent microscopy demonstrating localization to viral replication sites. Overall, probing the local protein environment of dysregulated lipids using functionalized lipid analogues allowed us to identify novel cellular factors promoting Zika virus replication. Ongoing studies aim to further clarify the role of top hits in the Zika virus life cycle and to assess their interaction with individual sphingolipid species.

#26 - SMAP3-ID to Map Native Protein–Protein Interactions of the Nuclear Lamina

Dr. Julia Warren, OHSU
Dr. Jian Wang, OHSU
Dr. Francis Dhoro, OHSU
Dr. Bo Chao, OHSU
Dr. Ashok Reddy, OHSU
Prof. Stefanie Kaech Petrie, OHSU
Prof. Xiangshu Xiao, OHSU
Pro. Bingbing Li, OHSU

The network of assembled intermediate filament proteins along the inner nuclear membrane constitutes the nuclear lamina. As a structural component of the nuclear envelope, the lamina imparts the nucleus with resistance to mechanical stress. Beyond this structural role of the lamina, its component proteins, nuclear lamins (LA, LB1, LB2 and LC), participate in other essential nuclear signaling functions that include mechanosensing, DNA repair, chromatin regulation, gene transcription and stem cell regulation. Morphological changes to the nuclear lamina arising from genetic mutations and/or alterations to post-translational processing of lamins are associated with a number of diseases, including some forms of muscular dystrophies, lipodystrophies and Hutchinson-Gilford Progeria Syndrome. The disease-associated structural changes in the nuclear lamina are often associated with changes in protein-protein interactions at the nuclear lamina. Existing genetic and biochemical methods to interrogate the lamin-protein interactome typically disrupt the native cellular environment, altering any identified 'endogenous' interactome. To minimize the generation of these artifacts, we have developed a sequential, biorthogonal, small molecule-assisted method to capture and identify the lamin interactome. The development of small molecule tools to facilitate the identification of protein-protein interactions requires highly specific ligand-target binding. Here, we leverage the target specificity of Lamin Binding Ligand 1 (LBL1) and its corresponding photoaffinity probe LBL1-PCF to introduce a novel method of proximity labelling: small-molecule-assisted identification of protein–

protein interactions through proximity (SMAP3-ID). With SMAP3-ID, we discovered novel roles of LA in regulating mitochondrial metabolic activities (*JACS Au*, **2025**).

#27 - Autoantibody-bound NMDA receptor structures uncover antigenic hot spots in anti-NMDAR encephalitis

Dr. Junhoe Kim, Vollum Institute, Oregon Health and Science University
Dr. Farzad Jalali-Yazdi, Vollum Institute, Oregon Health and Science University
Dr. Brian E. Jones, Vollum Institute, Oregon Health and Science University
Dr. Gary L. Westbrook, Vollum Institute, Oregon Health and Science University
Dr. Eric Gouaux, Vollum Institute, Oregon Health and Science University, Howard Hughes Medical Institute

Autoantibodies targeting synaptic membrane proteins are associated with autoimmune encephalitis manifested by seizures, psychosis, and memory dysfunction. Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis, a prototype of these autoimmune synaptic disorders, is surprisingly common. Unfortunately, how the native repertoire of anti-NMDAR autoantibodies recognizes NMDARs and the precise locations of antigenic epitopes remain poorly understood. Here we used an active immunization model that closely mimics the human disease to immunize adult mice with intact GluN1/GluN2A receptors, resulting in fulminant autoimmune encephalitis. Serum was collected at 6 weeks post-immunization for single-particle cryo-EM of GluN1/GluN2A receptors complexed with purified polyclonal anti-NMDAR autoantibody fragments. Native autoantibodies recognized two distinct binding sites on the GluN1 amino-terminal domain, which we confirmed using monoclonal antibodies bound to native NMDARs purified from mouse brain. Structural analysis of autoantibody-bound NMDAR complexes identified antigenic hot spots within the GluN1 amino-terminal domain. These hot spots provide potential targets for therapeutic intervention.

#28 - Suboccipital Release Osteopathic Manipulative Technique: A Non-Invasive Method for Autonomic Nervous System Modulation

Maxim Crasta , Meritus School of Osteopathic Medicine,Hagerstown,MD
Ryan Witczak ,Lake Erie College of Osteopathic Medicine,Elmira ,NY
Jennifer Kachelmeyer,Lake Erie College of Osteopathic Medicine,Elmira,NY

Introduction & Background SOR technique promotes wellness and balances the Autonomic Nervous System (ANS) Targets mechanoreceptors in the deep fascia, connective tissue, and muscles of the sub occipital area Alleviates stress and improves blood flow in the suboccipital region Potentially relieves compression on the vagus nerve, enhancing vagal activity This study examines SOR's impact on cardiac control using QT variability as a surrogate measure. Hypothesis SOR targeting the upper cervical spine or suboccipital region may have a beneficial impact on the functioning of the parasympathetic division of the autonomic nervous system. This could aid in restoring normal homeostatic conditions and physiological balance, potentially leading to increased vagal activity towards the heart, and may be indicated by EKG variables such as QT variability. Methods or Approach 24 healthy adults (12 male & 12 female) Crossover design with three groups No physical contact group (control), sham treatment (sham), and suboccipital release (SOR) Results SOR targets mechanoreceptors in the suboccipital area's deep fascia, connective tissue, and muscles enhancing vagal activity With EKG variables, we wanted to explore the impact on the heart Data collection involved QRS, QT, QTcB, JT, QTa, and QTend intervals, electrophysiological balance (iCEB) calculated from QTcB and QRS duration SOR group showed significantly lower mean QTa values and a significant decrease in the QTend ratio compared to other groups SOR resulted in a moderate increase in QT and QTcB length, along with a decoupling of QTend and JT intervals,

leading to shortened QTend intervals potentially due to increased vagal activity Moderate increase in QTcB suggests that SOR may improve cardiac function by prolonging the effective refractory period and enhancing ventricular relaxation. Conclusions and Next Steps SOR manipulation results in shortened QTend intervals, potentially due to changes in repolarization or temporary heart rate decrease due to parasympathetic stimulation Post-SOR data shows increased parasympathetic activity, evidenced by decoupling of QTe from JT intervals and shorter QTend intervals, leading to a longer effective refractory period and improved ventricular relaxation, indicating potential cardiac benefits Advanced techniques using biomarkers of autonomic activity (e.g., heart rate variability, baroreflex sensitivity) may further characterize the cardiac effects of SOR.

#29 - Phosphorylation-Induced Monomerization of 14-3-3ζ Triggers Proteasomal Degradation in Human Cells

Ms. Moriah Mathis, Oregon State University
Dr. Phillip Zhu, Oregon State University
Dr. Yunan Zheng, AbbVie Inc.
Dr. Kedar Puvar, AbbVie Inc.
Dr. Becca McCloud, AbbVie Inc.
Ms. Lina Blanco, AbbVie Inc.
Dr. Rick Cooley, Oregon State University
Dr. Ryan Mehl, Oregon State University

Although 14-3-3 proteins are central regulators of cellular signaling, little is known about how their function is altered by phosphorylation at Ser58 and subsequent monomerization. This post-translational modification has been proposed to remodel 14-3-3's extensive interaction network, but its physiological consequences remain unclear. Using genetic code expansion (GCE) to site-specifically install the non-hydrolyzable analog of phosphoserine into 14-3-3ζ at S58, we discovered a phosphorylation-dependent interaction between monomeric 14-3-3ζ and cereblon (CRBN), the substrate receptor of a clinically important E3 ubiquitin ligase complex. This finding suggests that phosphorylated 14-3-3ζ may be directed to CRBN for ubiquitination and proteasomal degradation, or might recruit its own client proteins for selective turnover. To explore this mechanism, we delivered purified, Myc-tagged 14-3-3ζ variants into HEK293T cells via electroporation and monitored their stability by Western blot. Phosphorylated, monomeric 14-3-3ζ is degraded significantly faster than wild-type protein, consistent with regulated proteasomal turnover. Ongoing experiments are testing additional 14-3-3 isoforms and mutants to define the molecular basis of this phosphorylation-dependent degradation. Together, this work reveals a new connection between 14-3-3 proteins and E3 ligases that may provide insight into previously unrecognized mechanisms of ubiquitin-mediated protein degradation and lead to new chemical biology approaches for reprogramming proteostasis.

#30 - Tools to explore chemical glycoproteomics data

Kathryn Kothlow1, Vishnu R. Tejus1, Tim S. Veth1, Haley M. Schramm1, Emmajay Sutherland1, and Nicholas M. Riley1

Chemical glycoproteomics technologies have enabled the identification of specific glycosylation sites and glycan structures that modulate protein function in a number of biological processes. Advances in sample preparation and data acquisition strategies have driven concomitant improvements in software for analyzing glycoproteomics data. Despite the progress in the ability of modern search engines to assign glycopeptide identifications, the glycopeptide tandem MS spectra generated in these workflows are complex and often challenging to interpret. Careful evaluation of glycopeptide MS/MS spectra can provide important clues, but exploring well annotated spectra is not often automated. For example, glycan-specific fragments like oxonium ions can inform a number of features about glycans that modify glycopeptides, including incorporation of monosaccharide analogs and glycan class, composition, and structure. Oxonium ions are widely used to evaluate raw data, and most modern glycoproteomics search tools include

them in scoring functions when identifying their spectra. Despite their widespread utility, few tools exist for evaluating the oxonium content of (glyco)proteomic datasets, especially prior to database searching.

Here, we introduce GlyCounter, a tool to that extracts oxonium ion information from raw data files to provide a snapshot of glycoproteome content that can be valuable for subsequent method optimization and database searching. We provide several examples of how GlyCounter can aid glycoproteomics data analysis, including highlighting a currently unpublished dataset where we used Seer nanoparticles to enrich glycoproteins from biofluids like plasma and cerebrospinal fluid.

Furthermore, manual inspection of glycopeptide MS/MS spectra remains a cornerstone of accurate chemical glycoproteomics. Although modern search engine algorithms can identify intact glycopeptides, the intricacies of glycopeptides often lead to incorrectly annotated spectra. To address these challenges, we have extended the web-based Interactive Peptide Spectral Annotator (IPSA) into IPSA 2.0, an open-source platform that facilitates systematic manual glycopeptide annotation. By allowing researchers to verify proposed compositions, adjust ambiguous modifications, and confirm that each observed ion is accounted for, IPSA 2.0 bridges the gap between automated assignments and rigorous manual validation. In all, we present GlyCounter and IPSA as new tools to evaluate chemical glycoproteomic data, ultimately enabling method refinement in sample preparation, data acquisition, and post-acquisition identification."

#31 - Environment-sensitive and red light activatable cage for biological application

Dr. Pankaj Gaur, Université de Strasbourg, France
Ms. Chiara Gigliucci, Université de Strasbourg, France
Ms. Julie Obeid, Université de Strasbourg, France
Dr. Andrey Klymchenko, Université de Strasbourg, France

Photocaging/caging is a technique in which a molecule of interest can be rendered biologically inert by covalent attachment with a cage.[1] Illumination of the caged compound results in a rapid and repetitive concentration jump of biologically active molecule leading to photo-controlled biological effect, such as activation of cellular receptors.[1,2] Therefore, photomanipulation of cellular chemistry using caged compounds has emerged as a powerful technique to provide a highly precise and spatiotemporal control over the release of various biomolecules, second messengers and drugs.

Despite the great achievements of the cage compounds in the biological manipulations, the need of UV or violet light for their stimulations makes them incompatible with the applications requiring deep tissue penetration. Moreover, these cages are exclusively controlled by light, and hence suffer from the major issue of off-target biological effects. It necessitates further development to explore new chemical strategies to design cages capable of cell-specific and microenvironment-controlled photoactivation. Variation in the cellular microenvironment, namely polarity and viscosity, is the hallmark of several pathological conditions such as cancer and neurodegeneration. By exploiting these differences, new cages could recognize target cells and tissues, and thereby helping to minimize the off-target effects.

In this regard, we designed and synthesized a series of meso-methyl BODIPY-based cages. By exploiting the environmentally-regulated excites states of the solvatochromic probes (Fig.1),[3] we structurally engineered, for the first time, an environment-sensitive BODIPY cage through systematic tuning of its push-pull architecture. A comprehensive structural-activity relationship study including photophysical and photochemical investigations as well as kinetic and mass analyses were performed to demonstrate the influence of electron-donating substituents on the environment-sensitive photochemical performance of BODIPY cages. Among the synthesized compounds, one exhibited the microenvironment-enabled light up (fluorogenicity) accompanied by photorelease in

[1]G C R Ellis-Davies, *Nat. Methods.* 2007, 4, 619-628.
[2]P. Klan, *J. Chem. Rev.* 2013, 113, 119-191.
[3]A. Klymchenko, *Acc. Chem. Res*. 2017, 50, 366-375.

apolar media. These results indicate that improving push-pull properties of BODIPY cage enables environment-sensitive uncaging through the generation of a high energy excited state in apolar media (Fig.1). Taken together, environment-sensitive cage is expected to offer a modular design strategy for new photochemical tools for imaging and therapeutic applications in the field of photopharmacology.

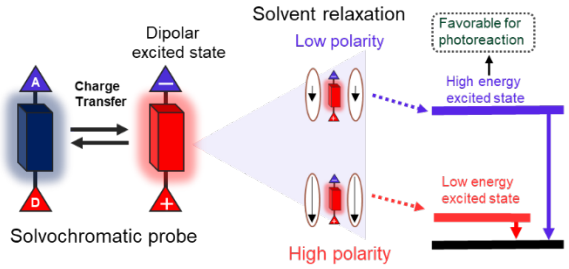


Fig.1: Schematic conceptualization of the environment-sensitive photocage.

Acknowledgement: *We greatly acknowledge the support of Marie Sklodowska-Curie Actions-2022 (Memcage-101110293) and UMR 7021 CNRS.*

#1 - Natural product-derived Ianthelliformisamines inhibit protein translation and block bacterial flagellum assembly

Mr. Max Bottlinger, Technical University of Munich
Dr. Martino Morici, University of Hamburg
Dr. Sophie Brameyer, Ludwig-Maximilians-University Munic
Ms. Elena Fajardo-Ruiz, Ludwig-Maximilians-University Munich
Ms. Isabella Gantner
Prof. Kirsten Jung, Ludwig-Maximilians-University Munich
Prof. Daniel Wilson, University of Hamburg
Prof. Stephan Sieber, Technical University of Munich

The escalating antibiotic resistance crisis is expected to threaten up to 10 million lives annually by 2050, underscoring the urgent need for novel antibacterial agents.[1] Natural products have long served as a rich source for drug discovery, and the characterization of naturally derived compounds enables access to novel modes of action (MoA).[2]

In this study, we investigated the MoA of the underexplored class of Ianthelliformisamines (Ian), which bear a Michael acceptor as an electrophilic warhead and exhibit activity against hard-to-treat Gram-negative bacteria such as Escherichia coli.[3] Using a combination of organic synthesis, chemical proteomics, and biochemical assays, we propose a dual MoA for Ian. Three Ian analogs and one chemical probe for activity-based protein profiling (ABPP) were synthesized. The probe was designed to retain antibacterial activity by substituting a methoxy group on the aromatic moiety with a propargyl ether, enabling covalent target capture via click chemistry. All derivatives demonstrated potent antibacterial activity in the presence of bicarbonate, a physiological component of human serum.

ABPP experiments identified infA, an essential translation initiation factor, as a target. In vitro translation assays confirmed concentration-dependent inhibition of protein biosynthesis by Ian derivatives. Additionally, fliC, a key component of bacterial flagella and a virulence-associated protein, emerged as a prominent hit in chemical proteomics. Recombinant Flagellin was labeled by the probe in a concentration-dependent manner, and motility assays revealed impaired bacterial movement upon Ian treatment. Indeed, Ian treatment blocks the assembly of the flagella as shown by transmission electron microscopy. Notably, Ian reduced the invasion of pathogenic E. coli into human host cells within its therapeutic window, highlighting fliC as a promising anti-virulence target for further development.

References:
[1] O'Neill J., Review on Antimicrobial Resistance, 2014. Available from: https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf
[2] Dias D.A., Urban S., Roessner U., Metabolites, 2(2):303-36, 2012.
[3] Khan et al., Org. Biomol. Chem., 12, 3847, 2014.

#2 - Effect of O-GlcNAcylation on α-crystallin aggregation

Ms. Michelle Marie Helmeke, University of Southern California
Ms. Rhianna Haynie-Cion, University of Southern California
Dr. Matthew R. Pratt, University of Southern California

α-crystallins are small heat shock proteins (sHSPs) that make up ~40% of human eye lens proteins. These proteins have an important role in maintaining the refractive properties of the eye lens and as molecular chaperone proteins to curtail lens protein unfolding and aggregation. α-crystallins are composed of two subunits, αA-crystallin and αB-crystallin in a 3:1 ratio, respectively. The αB-crystallin (αBC) subunit has been identified as a prevalent misfolded, aggregated protein in cataracts. The Pratt Lab has previously shown that O-GlcNAcylation of sHSPs near their IXI/V domain can disrupt the important IXI/V/ACD binding interaction in these proteins (Figure 1). In the crowded environment of the eye lens, the inhibition of this interaction could impact the stability of these proteins, contributing to protein misfolding, aggregation, and ultimately cataract formation. Here, we investigate the aggregation of the unmodified version of αBC compared to the O-GlcNAc-modified αBC at threonine 162. Using Ficoll 400 to mimic the highly crowded environment of the eye lens and a light scattering assay to monitor aggregation, we find that compared to the unmodified protein, gT162 αBC aggregates more quickly and with a higher

intensity. These results show that increased O-GlcNAc levels in eye proteins could contribute to the formation of cataracts. Further research is being conducted to determine whether O-GlcNAc modifications disrupt the ability of αA-crystallin (αAC) to act as a chaperone to prevent α-crystallin aggregation.

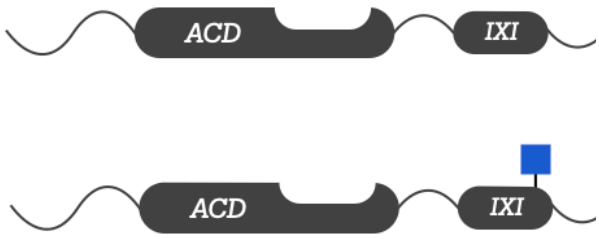


Figure 1. Interaction of IXI domain and ACD domain of sHSPs is disrupted by O-GlcNAc, denoted by a blue square

#3 - Discovery of Pyrroloquinazolines as EWSR1-ATF1 Inhibitors

Dr. Sridharashanmukharao B, Oregon Health & Science University, Portland
Mr. Jake Piesner, Oregon Health & Science University, Portland
Mr. Jingjin Chen, Oregon Health & Science University, Portland
Prof. Xiangshu Xiao, Oregon Health & Science University, Portland
Dr. Bingbing X. Li, Oregon Health & Science University, Portland

Department of Chemical Physiology and Biochemistry, Knight Cancer Institute, Oregon Health & Science University, Portland, OR97239.

Clear cell sarcoma of soft tissue (CCSST) is a rare soft tissue sarcoma with extremely poor prognosis. Currently there are no cures for CCSST. The defining feature of CCSST is the presence of fusion of the Ewing sarcoma gene EWSR1 with activating transcription factor 1 (ATF1) by chromosomal translocation. The EWSR1-ATF1 fusion is constitutively active in driving the transcription of genes that are normally regulated by ATF1 through phosphorylation. Genetic studies have convincingly demonstrated that EWSR1-ATF1 is critical for the development and maintenance of CCSST, suggesting that EWSR1-ATF1 is a promising target for developing targeted therapies for CCSST. However, no such drugs have been identified. We conducted a medium throughput screening to identify potential inhibitors of EWSR1-ATF1-mediated gene transcription. From this screening, we identified substituted pyrroloquinazolines as potent inhibitors of EWSR1-ATF1 without affecting wild type ATF1. Further structure-activity relationship (SAR) studies identified 755-8 as a novel potent inhibitor of EWSR1-ATF1 with strong growth inhibition activity in CCSST cells both in vitro and in vivo.

#4 - Synoviolin as a Novel E3 Ligase for PROTAC Development

Dr. Anandarao Munakala, Oregon Health & Science University
Dr. Julia Warren, Oregon Health & Science University
Dr. Bingbing X. Li, Oregon Health & Science University
Prof. Xiangshu Xiao, Oregon Health & Science University

Proteolysis-targeting chimeras (PROTACs) rely on E3 ligase recruitment for targeted protein degradation. Yet only a limited number of E3 ligases have been hijacked for PROTAC development. In this study, we aimed to expand the PROTAC toolbox by identifying novel E3 ligase ligands. Through systematic screening, we identified an aromatic acid **CF-1** as a novel small molecule ligand to Synoviolin (HRD1), a RING-type E3 ligase central to the endoplasmic reticulum-associated degradation (ERAD) pathway, as a promising candidate. To investigate if **CF-1** can be deployed for PROTAC development, we designed and synthesized JQ1-based conjugates with **CF-1** through both flexible and rigid linkers. These conjugates were evaluated for degradation of bromodomain-containing protein 4 (BRD4) to define the structure-activity relationship (SAR) of BRD4 degradation. Further mechanistic investigations demonstrated that the **CF-1**-based PROTACs indeed degraded BRD4 through PROTAC mechanism. Collectively, our findings highlight Synoviolin as a novel viable E3 ligase for

PROTAC development and support the potential of **CF-1** ligand in targeted protein degradation.

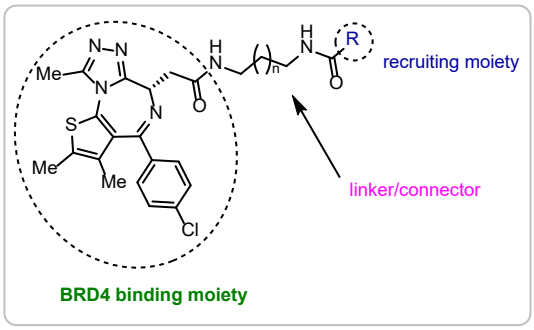


Fig. Design of JQ1-conjugated PROTACs

#5 - Exploring Intracellular Protein-Ligand Interactions with High Throughput Sequencing

Angelina Zhang, University of Washington
Dr. Dustin Maly, University of Washington

Significant effort has been made in screening small molecule interactions with proteins to find ligandable sites in proteins to develop therapeutic targets. However, we still lack an understanding of the molecular determinants of small molecule ligand binding. For instance, while large scale small molecule screens have been able to identify ligandable sites, it is still difficult to map the actual binding pocket, understand allosteric networks perturbed by the liganding event, and determine cellular responses to the chemical modulation. Therefore, we require tools to quantitatively study protein-ligand interactions in a native cellular context. Small molecule fragments (<300 Da) are able to target shallow binding pockets where allosteric regulation and protein-protein interactions occur, allowing us to access ligandable sites outside of deep binding pockets. Therefore, we aspire to develop an assay that is able to measure sensitive and selective liganding events of small molecule fragments and determine how the liganding event perturbs protein structure and function. Here, we present the development of a high throughput sequencing ligand screening method via LABEL-seq. LABEL-seq allows us to tag a protein with a RNA barcode. The protein and barcode are co-enriched via an affinity enrichment, and the barcodes are sequenced and related back to its assigned protein. Since sequencing is sensitive and precise, we can utilize LABEL-seq as a platform to profile covalent ligands-protein interactions. Here we show that we can profile proteins at low concentrations through a gain of signal assay, giving us selective protein-ligand pairs. Additionally, we anticipate measuring the liganding event across a deep mutational scan will allow us to elucidate the structural elements of small molecule protein binding interactions, understand drug pharmacology effects across specific variants, and predict allosteric networks. This will enable us to create a structure-function map in relation to protein ligand interactions on an amino acid resolution. By furthering our knowledge of small molecule and protein interactions and effects, we can better understand mechanistic, structural, and functional impacts of liganding a protein, which can help inform therapeutic drug development.

#6 - Portable and scalable profiling of protein variant libraries with LABEL-seq

Madeline Walsh, University of Washington
Dr. Jessica Simon, University of Washington
A. Felicia Adebanjo, University of Washington
Dr. Raining Wang, University of Washington
Melinda K. Wheelock, University of Washington
Allyssa Vandi, University of Washington
Aman Shihora, University of Washington
Dr. Sriram Pendyala, University of Washington
Dr. Daniel Holmes, University of Washington
Abigail McGee, University of Washington
Melissa Hopkins, University of Washington
Jordan Opsahl, University of Washington
Prof. Douglas M. Fowler, University of Washington
Prof. Dustin J. Maly, University of Washington

Multiplexed assays of variant effects (MAVEs) use high-throughput sequencing to profile the abundance, interactions, and regulation of thousands of protein variants in a single, pooled experiment. The regulation and function of many proteins rely on intracellular partners; as such, functional profiling in a native cellular environment is crucial to fully understand variant effects. LABEL-seq (Labeling with barcodes and enrichment for biochemical analysis by sequencing) enables large-scale profiling of biochemical and functional properties of protein variant libraries in mammalian cells by exogenously expressing a self-assembling RNA binding protein and circular RNA (circRNA) barcode. Previously, library generation in mammalian cells for LABEL-seq required a genomically integrated dual RNA Pol II and Pol III

promoter landing pad for bi-transcript (BiT) expression to enable independent expression of a protein variant and circRNA barcode, respectively. However, tandem expression of both the protein variant coding sequence and circRNA barcode in a single transcript (SiT) would allow for LABEL-seq portability into any existing RNA Pol II mammalian cell exogenous gene expression system. Here, we present the tandem expression of both the protein variant and circRNA barcode from a single RNA Pol II promoter. By leveraging stabilizing RNA structural elements, a marked increase in circRNA barcode concentration was achieved while maintaining protein expression, allowing for miniaturization of LABEL-seq assays. Additionally, we demonstrate that LABEL-seq can be used to assay toxic transgenes when single transcript expression is introduced in a dual RNA Pol II promoter landing pad cell line. We use SiT expression to profile the abundance of a site saturated mutagenesis library of the oncoprotein KRas, providing insight into the stability of KRas variants.

#7 - High-throughput Covalent Fragment Screening to Develop Selective Inhibitors and probes for Bacterial and Viral Hydrolases

Dr. Tulsi Upadhyay, Stanford University
Dr. Chenzhou Hao, Stanford University
Dr. Chieh Wen Lo, Stanford University
Dr. Stephen D. Ahator, The Arctic University of Norway
Dr. Md Jalal Uddin, The Arctic University of Norway
Dr. Daniel Abegg, University of Illinois Chicago
Dr. Emily C Woods, Stanford University
Dr. Christian S. Lentz, The Arctic University of Norway
Prof. Alexander Adibekian, University of Illinois Chicago
Dr. Matthias Fellner, University of Otago, Dunedin, New Zealand
Prof. Shirit Einav, Stanford University
Prof. Michael Lin, Stanford University
Prof. Matthew Bogyo, Stanford University

High-throughput Covalent Fragment Screening to Develop Selective Inhibitors and probes for Bacterial and Viral Hydrolases Tulsi Upadhyay1, Chenzhou Hao2, Chieh Wen Lo3, Stephen D. Ahator4, Md Jalal Uddin4, Daniel Abegg5, Emily C Woods1, Christian S. Lentz4, Alexander Adibekian5, Matthias Fellner6, Shirit Einav3, Michael Lin2 and Matthew Bogyo1,7

1Department of Pathology, Stanford School of Medicine, Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA 2Department of Neurobiology, Stanford University, Stanford, CA 94305, USA, Department of Bioengineering, Stanford University, Stanford, CA 94305, USA and Department of Chemical and Systems Biology, Stanford University, Stanford, CA 94305, USA 3Department of Medicine, Division of Infectious Diseases and Geographic Medicine, and Department of Microbiology and Immunology, Stanford University, California, USA 4Research Group for Host-Microbe Interactions, Department of Medical Biology and Centre for New Antibacterial Strategies (CANS) UiT, The Arctic University of Norway, 9037 Tromsø, Norway 5Department of Chemistry, University of Illinois Chicago, Chicago, Illinois, 60607 USA 6Biochemistry Department, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand 7Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA, USA E-mail: tulsiu@stanford.edu In the last decade, covalent inhibitors have gained popularity in drug discovery, with nearly 30% of newly approved drugs having a covalent mechanism of action. The recent success of the covalent drug nirmatrelvir, which targets the SARS-CoV-2 protease, and the β-lactam antibiotic class, which targets penicillin-binding proteins in bacteria, demonstrates the potential of the covalent inhibition strategy. These inhibitors offer significant advantages over noncovalent counterparts, including improved selectivity, prolonged target engagement, reduced dosing frequency, and a lower chance of developing resistance. In addition, covalent inhibitors can be used to generate activity-based probes for imaging and functional studies of enzyme targets. However, most reported covalent inhibitors have been generated from existing, well-established core scaffolds and electrophiles, emphasizing the need for innovative discovery approaches to identify new classes of small-molecule covalent binders. To address this gap, we employed a library of serine and cysteine-targeted covalent fragments to perform inhibition screens for multiple pathogen-derived hydrolase targets. This strategy uses low-molecular-weight (<500 Da) electrophilic fragments attached to a diverse range of

covalent electrophiles to identify potent hits directly from primary screening. We applied this approach and identified potent and selective covalent binders for three functionally important serine hydrolases from Staphylococcus aureus, as well as for two viral cysteine proteases. By coupling fragment screening with enzyme activity–based assays and mass spectrometry, we identified electrophile scaffolds that covalently modified the intended targets with nanomolar potency without the need for extensive medicinal chemistry. Structural analysis identified unique binding modes of the inhibitors, and we used the resulting inhibitors to assess the effects of selective inhibition of target hydrolases on virulence and biofilm formation. Similarly, we identified inhibitors of viral targets with sub-micromolar antivirulence activity. Derivatization of these lead molecules with fluorescent tags yielded highly selective fluorescent imaging probes that could be used to track the viral protease localization during the infection cycle in the host cell. Overall, our work demonstrates that focused covalent fragment screening is a powerful tool for rapidly identifying highly potent and selective electrophiles targeting essential enzymes in diverse pathogens.

#8 - Engineering Manganese MOFs for MRI-Active siRNA Delivery

Ms. Rachel Dunne, Oregon State University
Mr. Jacob Lessard, Oregon State University
Dr. Dan Liefwalker, Oregon State University
Dr. Kyriakos Stylianou, Oregon State University

Short interfering RNA (siRNA) is a well-known and powerful mechanism for regulating gene expression in cells. However, there are significant barriers to delivery, particularly in difficult-to-transfect cancers such as leukemia and other blood cancers, which exhibit poor uptake efficiency and endosomal escape. Because of this, typical transfection reagents which promote formation of liposomes or micelles to deliver encapsulated siRNA through the bilayer are insufficient. Therefore, a different approach to delivery of gene-silencing sequences in these highly aggressive cancers is needed. Metal organic frameworks (MOFs), which are composed of metal ions and organic ligands that coordinate together to form three-dimensional networks, offer an exciting alternative to lipid-based transfection reagents. This is due to their capacity for surface functionalization and encapsulation of molecules. This study aims to develop a novel manganese-based MOF for siRNA delivery. The MOF contains manganese ions which have 5 unpaired electrons, making it paramagnetic. This paramagnetism allows the MOF to be used as an MRI contrast agent, enabling non-invasive cellular tracking of the MOF-siRNA delivery vehicle. As a proof of concept, this MOF was tested in GFP-expressing HEK293T cells to establish a baseline efficacy and optimize the delivery mechanism under controlled conditions. Results showed efficient siRNA encapsulation into the MOF. Successful demonstration of GFP silencing with this MOF-siRNA complex will provide a foundation for future applications of theranostic MOF delivery systems in oncology.

#9 - Strategies for Molecular Glue Discovery

Dr. Kedar Puvar, AbbVie, Inc
Dr. Yunan Zheng, AbbVie, Inc
Dr. Justin M. Reitsma, AbbVie, Inc

Molecular glues represent a new frontier in therapeutic research, enabling targeted protein degradation. These small molecules induce protein-protein interactions that facilitate the ubiquitination and subsequent degradation of unwanted proteins. Their ability to selectively modulate protein function through degradation rather than inhibition provides a novel strategy to tackle previously undruggable targets and their compact size offers superior physicochemical properties to heterobifunctional degraders such as PROTACS.

In this abstract, we explore the strategies employed in discovering molecular glue degraders including efforts from AbbVie's Protein Degradation Platform Team and external collaborations. Our discussion involves a targeted approach incorporating bioinformatics to identify specific targets to degrade.

A prime example is the exploitation of Cereblon (CRBN) interactions—utilizing the G-Loop motif to discover putative neosubstrates. Recently, multiple groups have reported mining the structural proteome to find G-loop motifs that resemble those of the known CRBN neosubstrate CK1alpha. Here, we expand on this

approach and find that utilizing multiple neosubstrates as search templates provide us with additional information on gluable targets.

By integrating these strategies, we aim to propel the discovery and development of molecular glues, enhancing our capacity to tackle challenging therapeutic targets with precision and efficacy.

All Authors were or are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication. No honoraria or payments were made for authorship.

#10 - Building a more versatile genetic code expansion system for encoding phospho-Threonine

Sarah Louie, Oregon State University
Davis Sharts, Oregon State University
Hung Nguyen, Oregon State University
Russell McLoughlin, University of Oregon
Dr. Parisa Hosseinzadeh, University of Oregon
Dr. Ryan Mehl, Oregon State University
Dr. Rick Cooley, Oregon State University

Recombinant production of site-specifically phosphorylated proteins for in vitro characterion remains a major technical barrier to understanding how individual phosphorylation events regulate signaling and function. While genetic code expansion (GCE) enables translational incorporation of post-translational modifications such as phosphoserine (pSer) and phosphothreonine (pThr), the current systems for pThr suffer from low efficiency and mis-incorporation of competing amino acids. These limitations have prevented generation of biologically relevant, stoichiometrically phosphorylated proteins. We have now developed a clean and efficient pThr GCE system by improving intracellular pThr bioavailability and optimizing E. coli host cell lines to eliminate pSer and natural amino acid mis-encoding. Our approach integrates screening of pduX (a free threonine amino acid kinase) orthologs to enhance pThr biosynthesis and systematic tuning of translational components to increase encoding fidelity. Building on this platform, we are applying structural analyses, AI-guided active-site library design, and directed evolution to further enhance pThrRS enzymatic activity. Together, these innovations provide more effective tool for introducing phosphothreonine into proteins in order to conduct detailed mechanistic studies of threonine phosphorylation in signaling and enzymatic regulation.

#11 - Chemical genetic approach for cell-selective identification of glycosylation

Mr. Ramon Gudino Garcia, University of Southern California
Dr. Giuliano Cutolo, Abbott laboratories
Dr. Moira Morales, University of California, Irvine
Mr. Jackson Bishop, Abbott laboratories
Mr. Andrew Condon, University of California, San Francisco
Mr. David Hovanesyan, University of Southern California
Mr. Antonio Ramirez, University of Southern California
Ms. Manta Sinhaphalin, University of Southern California
Prof. Matthew Pratt, University of Southern California

Glycosylation of proteins is arguably the most diverse and most abundant post-translational modification. The end products of glycosylation serve important functions in metabolism regulation, signal transduction, cell recognition, and ageing. The disruption of this modification is linked to different health problems, including cancer, diabetes, heart diseases, and neurodegenerative diseases. Therefore, there is great interest in developing tools to study the biochemical mechanisms and participating enzymes in this protein modification to develop new therapeutics. With this regard, Metabolic Chemical Reporters (MCRs) are glycan-based probes for identification and visualization of glycans in the cell. These glycan MCRs are analogs of the naturally occurring monosaccharides that form the glycans in the cell (therefore can be used by naturally occurring enzymes to construct the glycans in the cell), with the exception that these analogs bear bioorthogonal reactive groups for subsequent conjugation to visualization and/or identification probes. Many of the current MCRs, however, cannot differentiate between cell types and populations, which is a limitation of this strategy when studying glycosylation in a mixed population of cells. Here, I present a chemical-

genetic approach to render these MCRs suitable to study glycosylation in a co-culture containing two or more cell populations. In this approach, we designed a caged MCR probe that is only active and tags proteins in the presence of a specifically engineered decaging nitroreductase. After treatment with the caged MCR probe of a co-culture containing both wild-type cells and cells transfected to express the nitroreductase, in-gel and microscopy fluorescence studies proved selective protein labelling only in the cells expressing the decaging nitroreductase, with no protein labelling in the wild-type cells. In the future, these caged MCRs could function as a strategy to study the implications of glycosylation in diseases with a complex cell environment, including cancer research in mice models.

Affiliation for all the authors at the time of the work: Department of Chemistry, University of Southern California

#12 - Chemoproteomic elucidation of β-lactam drug targets in Mycobacterium abscessus

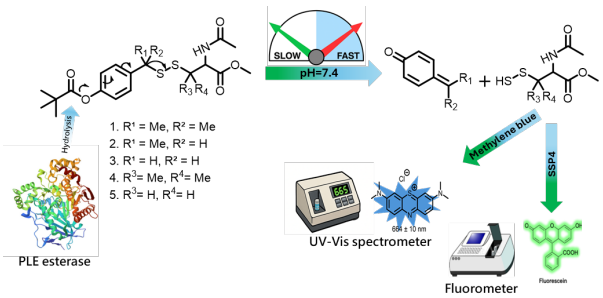
Dr. Kaylyn L. Devlin, Oregon Health & Science University
Emily Hutchinson, Oregon Health & Science University
Dr. Vivian Lin, Pacific Northwest National Laboratory
Dr. Gyanu Lamichhane, Johns Hopkins University
Prof. Kimberly E. Beatty, Oregon Health & Science University

Mycobacterium abscessus (Mab) is a pathogenic non-tuberculosis mycobacterium that causes severe and difficult to treat lung, skin, and soft tissue infections. Despite the rapidly rising incidence and clinical concern of Mab infections, treatment options remain limited and poorly efficacious. Treatment is complicated by Mab's ability to persist in a non-replicative, drug-resistant state. Some β-lactam antibiotics are potently bactericidal against Mab but are underutilized due to a lack of understanding of the molecular mechanisms of action against this pathogen. Using carbapenem activity based probes and chemoproteomics, we report the first comprehensive list of β-lactam target enzymes in Mab. We compared carbapenem targets across Mab subspecies as well as in actively replicating and non-replicative cultures. We report a new simple and accessible culture method for inducing a non-replicative state in Mab based on carbon starvation. Through these comparisons we identified 17 β-lactam targets that are active in each condition tested, seven of which were previously unknown to bind β-lactams. Finally, we characterized the β-lactamase activity and drug (imipenem and cefoxitin) inhibition profiles of nine targets. The findings presented advance our understanding of the mechanisms of action of carbapenems in Mab, a crucial step toward fully realizing the potential of β-lactams against this deadly pathogen.

#13 - Esterase-Responsive Persulfide Donors: Controlled Release and Detection

Dr. Ismail, University of Oregon, Oregon
Prof. Michael Pluth, University of Oregon, Oregon

Persulfides (RSSH) are reactive sulfur species (RSS) linked to hydrogen sulfide (H_2S) function and play key roles in vasorelaxation, memory, and the regulation of diseases like cardiovascular, neurodegenerative disorders, and cancer. The study of persulfides is challenging due to their dynamic equilibrium as well as their nucleophilic and electrophilic nature. Persulfide donors are needed to generate persulfides in situ to improve biological understanding. This research focuses on synthesizing esterase-activated persulfide donors for controlled release. Penicillamine-based persulfides were used as the releasing moiety, and structural modifications were explored to improve release efficiency. Bulky cumyl groups were found to enhance sulfur nucleophilicity, facilitating release of persulfide via ester hydrolysis leading to a self-immolative pathway. Kinetic data showed that Compound 1 ($k_{obs} = 7.43 \times 10^{-4}$ s^{-1}) reacted faster than Compound 5 ($k_{obs} = 2.06 \times 10^{-4}$ s^{-1}), indicating impact on release rate from the cumyl group. This study also investigates additional derivatives with methyl groups to assess their effect on release rates. The SSP4 probe and methylene blue assay were used to monitor the release of sulfane sulfur and HS^-, respectively. These methods allow for comparing release responses among various RSS species, offering insights into disulfide chemistry and aiding in the control of persulfide release. This work aims to enhance persulfide generation for potential future therapeutic applications.



#14 - Characterization and structure-activity-relationship analysis of ATP-competitive, conformationally selective apo-CDK2 inhibitors.

Sahiti N Shankar, University of Washington
Dr. Gayani K Perera, University of Washington
Dr. Sarvind Tripathi, University of California, Santa Cruz
Dr. Vishnu Kumarasamy, Roswell Park Comprehensive Cancer Center
Dr. Miklos Guttman, University of Washington
Dr. Erik Knusden, Roswell Park Comprehensive Cancer Center
Dr. Seth M Rubin, University of California, Santa Cruz
Dr. Dustin J Maly, University of Washington

Regulation of the cell cycle, particularly the transition from G_1 to S phase, is essential for controlled cell proliferation in response to growth factor signals and to enable cell quiescence or apoptosis under growth factor deprivation. This transition is governed by coordinated activity of CDK4/6–cyclin D and CDK2–cyclin E complexes, rendering CDK4/6 and CDK2 attractive therapeutic targets in cancer. Clinically approved CDK4/6 inhibitors effectively suppress proliferation, however, prolonged treatment frequently induces resistance through compensatory overexpression of cyclin E, which restores CDK2 activity and enables S-phase entry. Similarly, existing CDK2 inhibitors, such as PF-07104091 (PF4091), that engage the active, cyclin-bound conformation of CDK2 also trigger adaptive resistance via accumulation of cyclin E, which activates uninhibited apo-CDK2. While total inhibition of CDK2/4/6 is effective in inducing cell cycle arrest, it is not clinically viable, as it arrests proliferation in both malignant and healthy cells. Rather, an ideal pharmacological strategy would selectively stabilize apo-CDK2 in a conformation unfavorable for cyclin binding, thereby preventing reactivation of CDK2 signaling. Here, we report the discovery and characterization of a potent series of ATP-competitive inhibitors that are highly selective for apo CDK2 and are competitive with cyclin binding. Using structural, biophysical and biochemical analyses, we have profiled how this class of inhibitors affects CDK2–cyclin interactions, both in vitro and in the native mammalian cell context, and identified structural features critical for potency through structure–activity relationship studies. Finally, we demonstrate that the rebound in proliferation that is observed in cyclin-overexpressing cancer cells treated with inhibitors that target cyclin-CDK2 complexes can be prevented by co-administration of our selective apo-CDK2 inhibitor. Together, our results demonstrate that targeting apo-CDK2 is a promising strategy for treating cyclin-overexpressing cancers.

#15 - Photoswitchable Negative Allosteric Modulators at the Cannabinoid CB1 receptor

Mr. Jingte Ni, Oregon Health & Science University
Dr. Sajal Sen, Oregon Health & Science University
Prof. James A. Frank, Oregon Health & Science University

The endocannabinoid system is a lipid-based signaling network present across all vertebrate species, where it plays key roles in neuromodulation, pain, metabolism, and immune function. Among its components, the cannabinoid CB1 receptor (CB1R) is a G protein–coupled receptor (GPCR) highly expressed in the central nervous system and is responsible for regulating intracellular signaling pathways and synaptic transmission. Due to its broad physiological influence, CB1R has emerged as an attractive therapeutic target. However, conventional small-molecule ligands lack sufficient spatial and temporal precision to safely dissect receptor function, often resulting in undesirable off-target effects. Photopharmacology provides a promising strategy to overcome these limitations by integrating light-responsive motifs into pharmacologically active ligands. This work presents the synthesis of novel azobenzene-containing derivatives of the CB1R negative allosteric modulators PSNCBAM-1 and GAT-358, which are drug candidates for treating addictive disorders. Irradiation enables reversible

probe isomerization between trans and cis states, thereby allowing optical control over ligand affinity and efficacy at CB1R. With precise illumination, this platform enables us to have high-resolution spatiotemporal interrogation of CB1R activity. The photophysical properties of these compounds were characterized by using UV–Vis spectroscopy, confirming efficient and reversible photoisomerization when irradiated with 365-500nm lights. And we plan future applications in cells with FRET or GRAB eCB biosensors to evaluate their binding efficacy to CB1R and functional responses to drugs. The development of these photoswithable modulators establishes a novel paradigm for studying endocannabinoid signaling dynamics and may ultimately facilitate safer, more selective cannabinoid-based therapeutic strategies. Collectively, this work highlights the potential of photopharmacology to reshape drug design for neuromodulatory GPCR targets.

#16 - Platinum(II) and Psoralen-Based Crosslinking Tools Reveal Tertiary Structure and Long-distance Interactions in Viral RNA

Ethan Kimmett, University of Oregon
Dr. Dillon Willis, University of Oregon
Prof. Victoria DeRose, University of Oregon

Understanding the structural organization of viral RNA genomes is essential for elucidating mechanisms of replication, translation, and host regulation. The genomes of RNA viruses often form extensive secondary and tertiary structures that coordinate viral replication and transcription. Current computational methods of determining three-dimensional (3D) models remain challenging due to limited experimental information on long-range RNA-RNA contacts. To address this gap, our work develops new chemical and photochemical crosslinking approaches to experimentally define tertiary interactions, with application to the SARS-CoV-2 genome. We build on prior findings that platinum(II) complexes such as cisplatin can serve as effective and selectively reversible RNA crosslinkers. We have optimized reaction conditions for both native and modified platinum(II) reagents, including azide-functionalized analogs enabling downstream capture through biotin-DBCO conjugation, for use in cell-derived RNA lysates. We have also applied in-cell crosslinking with psoralen derivatives. Complementary to this chemical approach, we have developed a high-intensity, thermally regulated ultraviolet crosslinking apparatus enabling uniform irradiation and precise control over exposure parameters. This custom instrument enables rapid photo-induced RNA crosslinking with improved yield and reproducibility relative to conventional UV transilluminators. To capture long-distance interactions within viral RNA genomes, a next-generation sequencing (NGS) pipeline has been developed that captures both junction reads arising from covalent RNA–RNA adducts. Additionally, a novel NGS method enabling direct detection of platinum(II) RNA crosslink sites has been developed. Computational analysis of these junctions identifies candidate tertiary contact sites, revealing both previously predicted and novel interactions within the 5′ and 3′ untranslated regions (UTRs) of the SARS-CoV-2 genome. These experimentally derived constraints can be integrated into 3D modeling frameworks to refine global RNA architectures and assess how UTR conformations influence viral transcription and replication. Together, these advances establish a hybrid UV- and platinum-based crosslinking platform that couples controlled photochemical and chemical reactivity with high-throughput sequencing readout. The system provides robust, modular tools for mapping tertiary RNA contacts at transcriptome scale and can be readily extended to the structural characterization of emerging SARS-CoV-2 variants and novel RNA viruses. Beyond virology, the combined hardware and chemical toolkit offers a generalizable route for probing RNA structure–function relationships in diverse biological contexts.

#17 - Unveiling the Relationships between Nitration and Phosphorylation Using Genetic Code Expansion

Ms. Sarah B. McGee, Oregon State University
Dr. Stanislau Stanisheuski, Oregon State University
Dr. Richard B. Cooley, Oregon State University
Dr. Ryan A. Mehl, Oregon State University

Post-translational modifications (PTMs) are widespread throughout the human proteome. Among them, serine phosphorylation, which is catalyzed by kinases, and tyrosine nitration, which results from oxidative stress, are closely

associated with diseases such as ALS and cancer. These modifications often occur on the same protein, making it essential to understand whether and how phosphoserine and nitrotyrosine influence each other. In this work, we first optimize methods for efficient site-specific incorporation of nitrotyrosine into the model protein sfGFP. We then apply these methods to encode nitrotyrosine at defined positions in ERK, a protein whose nitration has been linked to cancer-related signaling pathways. Next, we use genetic code expansion (GCE) to incorporate phosphoserine into MEK, generating its active kinase form, and assess its ability to phosphorylate various nitrated ERK variants. Our results show that ERK nitration alters both its MEK-mediated activation and autophosphorylation kinetics, revealing a previously unrecognized nitration-dependent regulation of phosphorylation. Finally, we establish a dual-encoding system that enables precise, site-specific incorporation of both nitrotyrosine and phosphoserine within the same protein—representing the first example of a protein simultaneously modified by both PTMs through GCE. Together, these findings uncover a functional interplay between nitration and phosphorylation and suggest a potential mechanistic connection in their regulatory roles.

#18 - Optical Control of Cannabinoid Receptor Signaling: A Membrane-Tethered Photopharmacological Approach

Dr. Sarahi Garza, Oregon Health & Science University
Dr. James Frank, Oregon Health & Science University
Dr. Janelle Tobias, Oregon Health & Science University

Marijuana acts on the CB1 receptor and can be used medicinally for ailments such as pain, but poses risks due to its major psychoactive compound, □9-tetrahydrocannabinol (THC). CB1 is densely expressed in the brain and important for central nervous system function, but drug development has been hindered by our lack of understanding of circuit and tissue specific CB1 function. To better understand CB1 signaling researchers have also aimed at exploring endogenous cannabinoids like anandamide (AEA). However, the lipophilicity of cannabinoids limits their solubility in tissue and spatiotemporal control. Approaches like chemogenetics and optogenetics offer spatiotemporal control of neural circuits but they do not target endogenous CB1 signaling. In contrast, Photopharmacology uses light sensitive compounds, photoswitches and photocages to optically control receptor signaling with high temporal precision. However, currently existing photoactivatable cannabinoids lack the spatial selectivity needed to control CB1 on specific cell membranes or neural circuits. We were able to use a membrane-tethered approach with self-labeling proteins to design the first membrane-anchored photoswitchable THC compound, PORTL-THC, and the first membrane-anchored caged AEA compound, hOCT-AEA. Here we use Neuro2a cells and show that PORTL-THC can control endogenous CB1 signaling in a reversible manner. We also demonstrate that hOCT-AEA can activate an endocannabinoid sensor, GRABeCB2.0, with high spatiotemporal precision. Both tools overcome current limitations in the application of cannabinoid ligands and advance our ability to optically control CB1 on select cells.

#19 - Molecular Tools for Precision Imaging of Membranes

Ms. Paige Ring, University of New Hampshire
Mr. Tom Diphillippo, University of New Hampshire
Ms. Saghar Jarollahi, University of New Hampshire
Dr. Aakriti Garg, University of New Hampshire

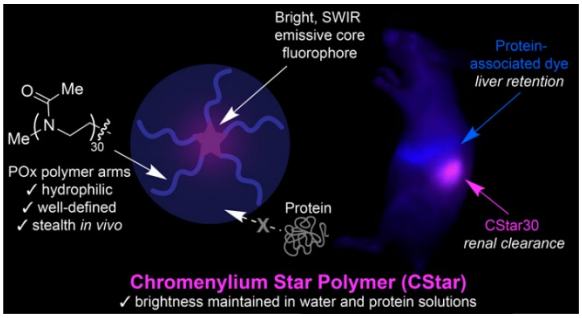
Cellular membranes act as physical barriers in cells that allow a multitude of biochemical process to occur simultaneously that are essential for life. The morphology of a membrane is directly coupled to its function and disruption of membrane structure has been associated with several human disorders. Mechanisms of non-apoptotic regulated cell death (RCD), that are characterized by changes in membrane morphology, have emerged as critical pathways contributing to the progression and treatment of diseases including cancer, and neurodegenerative and autoimmune disorders. Consequently, methods to characterize membrane morphology during these events are critical to fully understand and predict cellular outcomes. We present strategies to characterize membranes during RCD using activity-based sensors (ABS) and novel, viscosity sensitive fluorophores. ABS reagents that specifically detect hydrogen peroxide (H2O2) and labile iron

(Fe2+), potent signaling molecules that have been implicated in several RCD mechanisms, are anchored to membranes for characterization of morphology. Viscosity sensitive fluorophores are targeted to bilayers to act as reporters of membrane order during initial phases of RCD. Progress towards these research goals and insights gained regarding the influence of membrane morphology on the execution of specific RCD mechanisms, will be described.

#20 - Chromenylium Star Polymers Enable Sensitive, Targeted Fluorescence Imaging in the Shortwave Infrared

Emily B. Mobley, University of California Los Angeles (California, US)
Eric Y. Lin, University of California Los Angeles (California, US)
Dr. Merle M. Weitzenberg, National Center for Tumor Diseases (Dresden, Germany)
Kalina Bamberger, National Center for Tumor Diseases (Dresden, Germany)
Giuseppe Balsamo, National Center for Tumor Diseases (Dresden, Germany)
Julia Correa Remus, National Center for Tumor Diseases (Dresden, Germany)
Simon Härtl, National Center for Tumor Diseases (Dresden, Germany)
Prof. Oliver T. Bruns, National Center for Tumor Diseases (Dresden, Germany)
Prof. Ellen M. Sletten, University of California Los Angeles (California, US)

Optical imaging utilizing fluorophores as contrast agents has revolutionized the way we study biological processes in cells and transparent organisms, facilitating the advancement of biomedicine. Although optical imaging has many advantages such as low cost, fast speed, high resolution, and safe/non-toxic nature, there are challenges effectively translating these advantages to humans. This is because imaging more complex organisms suffers from significant background autofluorescence of endogenous emitters, light scattering and limited tissue penetration. Taking advantage of the low energy wavelengths of the shortwave infrared (SWIR, 1000–2000 nm), we are able to achieve substantial penetration of light through tissue with decreased scattering. *Unfortunately, the vast majority of small molecule organic SWIR contrast agents are extremely hydrophobic, displaying poor water solubility and non-emissive aggregation* in vivo, *which limits their photophysical properties, bioavailability, and most importantly, clinical applications.* This research merges biocompatible, stealthy poly(2-methyl-2-oxazoline) polymers with chromenylium-based SWIR fluorophores, resulting in a star polymer architecture– "Chromenylium star" polymers, or "CStar". CStars are well-defined SWIR fluorescent molecules that overcome these limitations. With the hydrophilic polymer arms acting as a pseudo-molecular shield for the hydrophobic fluorophore core, CStars are among the few organic SWIR dyes with high fluorescence quantum yields in water. Additionally, in contrast to water-soluble small molecule dyes, CStars experience minimal biomolecule interactions, facilitating efficient renal clearance within 1 day post-injection. Imbuing targeting ligands has further advanced the CStar fluorophore scaffold, enabling more sensitive, accurate imaging of disease states *in vivo*. Taken together, these SWIR-emissive materials are modular, require no additives, and can be targeted to disease-associated epitopes, thereby facilitating new diagnostic procedures, or helping surgeons selectively identify and remove diseased tissues.



#21 - Aminoacyl-Based Affinity Purification Enables Mapping of Host-Driven Modifications on Functional tRNAs for Genetic Code Expansion

Mr. Patrick Allen, Oregon State University
Dr. Richard Cooley, Oregon State University
Dr. Ryan Mehl, Oregon State University

Transfer RNAs (tRNAs) are increasingly recognized as therapeutic agents for treating genetic diseases, and in synthetic cells for genetic code expansion. Their translational efficiency depends on precise post-transcriptional modifications that influence structure, half-life, and codon recognition. However, existing purification methods show bias against modifications, limiting

characterization and subsequent therapeutic applications. We developed a function-based method—GCE Pulldown—that isolates mature, folded, and aminoacylated tRNAs based on their charged amino acid. This modification-agnostic approach selectively enriches for functional tRNA species and overcomes challenges posed by low yield and modification-induced bias in traditional methods. Using this technique, we purified Methanomethylophilus alvus Pyl-tRNA from both bacterial and mammalian cells and performed comparative modification mapping. We show that host-derived modifications are incorporated, and that these modified tRNAs exhibit improved activity in cell-free translation systems. This method enables detailed interrogation of tRNA maturation and modification states in heterologous hosts, providing critical insights into the molecular determinants of GCE efficiency. Our work advances the ability to functionally characterize orthogonal tRNAs and optimize their use in synthetic biology and protein engineering applications.

#22 - Modified GFP chromophores as environment-sensitive fluorescent turn-on probes for metalloenzymes

Ms. Sky Price, The University of Texas at Austin
Ms. Lauren Kimberly, The University of Texas at Austin
Ms. Dorothea Hudson, The University of Texas at Austin
Mr. Seth Choi, The University of Texas at Austin
Mr. Guilherme Ragi-Berto, The University of Texas at Austin
Dr. Emily Que, The University of Texas at Austin

Small molecule fluorophores are advantageous tools for the study of proteins, as they ca be employed in live cells and only minimally perturb protein localization and function. Their sensitivity and signal-to-background ratios can be enhanced through the use of environment-sensitive scaffolds. These have widely been employed for targeting proteins, where fluorescence becomes enhanced (“turns on”) when the probe is encapsulated by the protein of interest. We report the development of a new environment-sensitive scaffold and its application for sensing metalloenzymes. We find this scaffold to yield much more consistent fluorescence responses than previously explored fluorophores, granting it a significantly greater measure of versatility without compromising its relative specificity. We then demonstrate the implementation of this probe for screening enzyme inhibitors and for reporting enzyme metalation state, both in vitro and in live cells.

#23 - Cell-to-cell proteome changes induced by Bcl-2-overexpression in single TNBC-cells

Dr. Stanislau Stanisheuski, Oregon State University, Corvallis, OR
Dr. Jacob Porter, Oregon Health & Science University, Portland, OR
Dr. Andrew Emili, Oregon Health & Science University, Portland, OR
Dr. Siva Kolluri, Oregon State University, Corvallis, OR
Dr. Claudia Maier, Oregon State University, Corvallis, OR

Triple negative breast cancer (TNBC) is known for having the worst prognosis among breast cancers and there are no efficient therapeutic options for it. Elevated Bcl-2 levels are observed in many cancers, including TNBC. Bcl-2 is a pro-survival mitochondrial membrane protein, a potential therapeutic target in cancer known for its antiapoptotic activity. We used previously generated and characterized stable Bcl2-protein overexpressing MDA-MB-231 cells(Bcl2) and their empty-vector expressing analog(231e). BCA assay and flow cytometry were used to evaluate protein content and cell size distributions. 1,5,10-cell and bulk samples were prepared in 384-well microplates as described previously using an HP D100 Single Cell Dispenser. Orbitrap Astral was used for obtaining deep single-cell proteome coverage. High-throughput quantitation with fast 5-minute gradient was performed on Orbitrap Fusion Lumos. Both instruments were operated with FAIMS interface and coupled to Vanquish Neo UHPLC. 3700 proteins were quantified in single-cell samples with <20% missing values with median CV values 33%, 11% and 9% for 1-,5-,10-cell samples, respectively. More than 99% of quantified proteins showed no significant change between cell lines. Out of 3755 proteins with <50% missing values in each group, the number of differentially expressed ($p<0.01$, $FC>1.5$) proteins in 10-cell samples was only 13. Contrary to expectations, almost all differential proteins have a well-established connection to metastasis in the literature, not apoptosis. In addition to standard statistical analysis, due to

low variance in the dataset, we were able to target the proteins responsible for Bcl-2-dependent cell-to-cell heterogeneity by analyzing the proteins/peptides with abnormal distributions in single-cell samples. This novel strategy highlighted in a fraction of studied cells a clear Bcl-2-dependent regulation of even more proteins related to metastasis.

#24 - Micromap (µMap) Photoproximity Labeling for Integrated Phenotypic Screening and Accelerated Prioritization of Targeted Protein Degrader Compounds

Dr. Steve Knutson, Princeton University
Dr. Danielle Morgan, Princeton University
Dr. Ryan McNamee, Princeton University
Dr. Jack Sloane, Bristol Myers Squibb
Dr. Jonathan Olsen, Bristol Myers Squibb
Steven Levine, Bristol Myers Squibb
Dr. Gabe Mintier, Bristol Myers Squibb
Dr. Monica Kwok, Bristol Myers Squibb
Dr. Payal Sheth, Bristol Myers Squibb
Prof. David MacMillan, Princeton University

Phenotypic screening is a powerful tool for discovering first-in-class medicines, but its potential is limited by the significant challenge of triaging hit compounds and deconvoluting their mechanisms. Similarly, targeted protein degradation (TPD) represents a powerful new therapeutic strategy but is constrained by its reliance on a small number of E3 ligases with limited capability to discover new degradation mechanisms within validated platforms. Here, we present an integrated platform that bridges phenotype and mechanism, enabling rapid prioritization and characterization of small molecule hits from high throughput screening with sequential proximity labeling technologies. First, immunophotoproximity labeling (µMapX) is used to profile drug-induced interactome changes of an endogenous protein target to rapidly triage hits displaying discrete or promising mechanisms. Second, photocatalytic µMap target identification (µMap TargetID) is used to characterize protein engagement for candidate compounds and provide orthogonal mechanistic insight. As a test case, we applied this platform to discover degraders of BTB and CNC Homolog 2 (BACH2), a key immunoregulatory transcription factor. This approach rapidly prioritized a lead compound scaffold that inhibits GSK3β to induce proximity between β-catenin and BACH2, leading to its degradation through a LON peptidase-dependent mechanism. Together, this integrated platform provides a generalizable strategy to accelerate drug discovery by de-risking and prioritizing phenotypic hits, discovering degraders with novel mechanisms, and uncovering therapeutically targetable biology.

#25 - Live imaging of astrocyte development at single-cell resolution using zebrafish Brainbow technology

Mr. Suhail Akram, Vollum Institute, Oregon Health & Science University, Portland, OR, USA
Prof. Tim Czopka, Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK
Prof. Kelly R. Monk, Vollum Institute, Oregon Health & Science University, Portland, OR, USA
Dr. Dongeun Heo, Vollum Institute, Oregon Health & Science University, Portland, OR, USA

Astrocytes exhibit remarkable morphological complexity, enabling them to perform critical roles in supporting neuronal circuit development and function. However, our understanding of how astrocytes develop and establish their complex morphology remains incomplete, partially because astrocyte development occurs during the early postnatal period in mammals when direct optical access to the CNS is limited. To address this limitation, we utilized the larval zebrafish spinal cord as a model for live imaging of developing astrocytes in vivo. Larval zebrafish are optically transparent and offer a well-defined timeline of astrocyte development. To achieve single-cell resolution of individual astrocytes and visualize astrocyte-astrocyte interactions, we employed the membrane-targeted Brainbow system (Palmbow) in combination with HaloTag technology to induce combinatorial expression of spectrally distinct fluorescent proteins in astrocytes. Using this approach, we have begun to investigate astrocyte-astrocyte interactions during development and explore how astrocytes competitively grow to establish their minimally overlapping territories and tile the CNS.

#26 - Uncovering the mechanism responsible for Glucagon-like peptide-1 receptor (GLP-1R) recycling

Ms. Tatum Weishaar, Oregon Health & Science University

Dr. Braden Lobingier, Oregon Health & Science University

Glucagon-like peptide-1 (GLP-1) is a glucagon precursor that has become a popular therapeutic tool for the treatment of type 2 diabetes and obesity due to its ability to increase insulin secretion and decrease appetite and food intake. GLP-1 exerts its effects by binding to a class B G protein-coupled receptor, the Glucagon-like peptide-1 receptor (GLP-1R). Once activated by GLP-1, GLP-1R recruits and activates the Gas G protein which promotes the production of cyclic adenosine monophosphate and stimulates the signaling cascade responsible for GLP-1's therapeutic effects. Following Gas activation, GLP-1R is internalized into the cell and trafficked back to the cell surface in a process known as recycling which allows for GLP-1R to regain responsiveness to GLP-1 on the cell surface, further promoting GLP-1R's signaling cascade and therapeutic effects. This makes GLP-1R recycling an attractive process to study and understand for the development of improved clinical GLP-1R agonists; however, the mechanisms underlying GLP-1R recycling are not understood. This project combines receptor mutagenesis with novel chemical biology techniques to uncover the recycling motif and protein complex responsible for GLP-1R recycling, and preliminary data indicates that GLP-1R recycles through a noncanonical mechanism. Understanding GLP-1R recycling would allow for the development of improved clinical GLP-1R agonists for the treatment of diseases like type 2 diabetes and obesity.

#27 - Photo-released Diacylglycerol Modulates Insulin Secretion of Min6 Beta Cells

Varik Harris, Oregon Health and Science University
Dr. Dmytro Yushchenko, Miltenyi Biotech
Dr. Carsten Schulltz, Oregon Health and Science University

The regulation of hormone secretion by beta cells is intensely complex. An understudied pathway that controls these events is that of diacylglycerol (DAG), a second messenger that is produced following GPCR and phospholipase C activation, crucial steps in generating calcium spikes that drive secretion events. DAG is a ligand for proteins with C1 domains, such as protein kinase C isoforms, that have been shown to influence insulin secretion. Published and unpublished work by the Schultz lab shows that Protein Lipase C activation in beta cells generates a calcium signal accompanied by a spike in DAG. A hypothesis suggests that DAG is limiting the calcium signal by regulating plasma membrane calcium channels essential for hormone secretion (Frank et al. 2016). Further, lasting elevation in DAG levels induced by uncaging DAG stopped calcium oscillations. Our goal is to closely link DAG levels to insulin secretion. Our preliminary work using photocaged DAG species suggests that DAG indeed limits insulin secretion. An ELISA to measure secreted insulin in cell supernatant and cell lysate after DAG photo-release showed differences in secreted and stored insulin when compared to control groups. This novel chemical biology approach paves the way to more detailed studies to better understand the roles signaling lipids play in the secretory events of beta cells.

#28 - Structure of wild-type human green cone opsin (GCO) yields insights into mechanisms underlying the rapid decay of its active, signaling state.

Ms. Weekie Yao, Oregon Health & Science University
Prof. Jonathan F.Fay, University of Maryland
Prof. David L. Farrens, Oregon Health & Science University

Visual opsins are a family of G protein-coupled receptors that sense light. Most animals have two types - rod and cone opsins. These opsins play different roles - rod opsin (rhodopsin, or Rho) enables vision under low light conditions whereas cone opsins enable color vision during bright light. Interestingly, both opsin types use the same ligand to detect light - a retinal ligand that is covalently attached to the receptor by a Schiff base (SB). Light absorption causes the retinal to isomerize, which then induces conformational changes in the receptor that initiate G protein signaling and visual phototransduction. Although rhodopsin and cone opsins share the same mechanism for light detection outlined above, their active, signaling states differ drastically in stability – after photo-activation, cone opsins decay rapidly (in seconds) whereas rhodopsin decays in minutes. These differences have been proposed to be important for the two types of visual opsins to function under different lighting conditions.

To understand what causes this difference in active-state stability, we solved the structure of active-state, wild-type human green cone opsin (GCOWT) in complex with G protein, and compared it to a similar structures of rhodopsin-G protein complexes1. Our structure reveals unique features that help explain the lower stability of an active cone opsin. These includes: 1) a larger 'hole' next to the retinal Schiff-base attachment, which could both facilitate retinal escape and water access; 2) a larger water channel and concentration of polar residues near the Schiff-base that could facilitate Schiff-base hydrolysis; 3) a potentially anionic residue, E102, which lies within ~3.6 Å of the Schiff-base in the active-state. We find mutating this highly conserved glutamate (E102Q) significantly slows down the active-state decay of GCO (~8X) and increases G protein activation. Further kinetic analysis of GCOE102Q and GCOWT implies that entropic factors are the main cause of the fast decay of GCOWT. Our results suggest these structural features in GCOWT likely facilitate its function under bright light - rapid retinal release from activated cone opsin help prevent signal saturation and enable rapid turnover of the receptor.

References:
1. Yao, Fay and Farrens, PNAS (in press).

#29 - Probing the biomolecular target of near infrared nerve-specific fluorophores

Mr. William Greer1, Mrs. Alannah Maher,1 Mr. Antonio Montaño1, Mrs. Dani Szafran-Reeder1, Mr. Anas Masillati1, Mrs. Katie Emberley2, Dr. Ben Emery2, Dr. Diego Jacho3, Dr. Connor Barth3, Dr. Lei G. Wang1,2, and Dr. Summer L. Gibbs1,4
1Department of Biomedical Engineering, 2Jungers Center for Neuroscience Research, 3Trace Biosciences, Inc., Beaverton, OR 97006 4Knight Cancer Institute, Oregon Health and Science University, Portland, OR, 97201.

Background

Iatrogenic nerve damage remains a significant surgical complication, often resulting in chronic pain and functional loss. Identifying and preserving nerves intraoperatively is inherently challenging due to their small size, low contrast under white light, and anatomical variability—especially in traumatic cases.1 Fluorescence-Guided Surgery (FGS) offers a promising solution to improve intraoperative visualization; however, its effectiveness is limited by the lack of specific contrast agents for functionally critical off-target tissue structures.2 While several cancer-targeted fluorophores are advancing clinically, nerve-specific imaging agents remain underdeveloped due to the lack of a near infrared (NIR) nerve-specific fluorophores.

Methods

A library of first-in-class near-infrared (NIR) nerve-specific fluorophores was recently designed for compatibility with existing FGS clinical systems.3 These small-molecule probes are applicable across multiple surgical disciplines involving both the peripheral and central nervous systems. The probe physicochemical properties were finely tuned for optimal clinical applicability prior to further investigation into their subcellular interactions. The work presented herein is focused on the recent progress towards elucidating the biomolecular binding mechanisms of a subset of nerve-specific phenoxazine-based fluorophores, with special emphasis on their potential use as biomarkers for assessing myelination.

Behavior of various fluorophore are quantified by in vivo and ex vivo fluorescence imaging for normal, genetic, and injury models. Bridging the gap between in vivo and ex vivo fluorescence is crucial to confirm conserved binding interactions, necessitating methodology optimization for tissue clearing, whole mount imaging, and tissue manipulation. Conserved binding between in vivo and ex vivo staining was validated using longitudinal imaging of coronal brain slices. Following intravenous probe administration and subsequent washout, the probe was reapplied topically, using representative wide-field and high-magnification fluorescence images for comparison. A spatial comparison with immunofluorescence panels informed by RNA sequencing and proteomics is ongoing.

The Myrf/Sox10 demyelinating Cre tamoxifen-inducible mouse system was employed to determine fluorophore behavior with clinically relevant demyelination; and enables targeted, time-controlled deletion of Myrf in oligodendrocyte-lineage cells via Sox10-driven CreERT2, inducing demyelination in response to tamoxifen.4

Results

These novel fluorophores reliably highlight nerve tissue in vivo and ex vivo for both peripherally and centrally myelinated structures, indicating a conserved binding mechanisms with utility across surgical procedures. Experimental findings support their utility for real-time intraoperative imaging of myelin integrity by fluorescence imaging. Chemical removal of lipids retains fluorophore staining while lipid based FluoroMyelin Green is attenuated, supporting protein binding hypotheses, with ongoing proteomics serving to further determine specific protein interactions. Furthermore, in vivo binding consistency amongst structural analogs facilitates robust structure activity relationship (SAR) investigations and future rational designs that are driven by improved understanding of the probes' specific biomolecular interactions.

Conclusion

The results presented herein highlight the potential of these NIR nerve-specific fluorophores to improve nerve visualization during surgery by acting as biomarkers for myelination. This effectively lays the groundwork for disease-specific imaging applications and the expansion of advance both clinical and research-based diagnostics and future rational design of small molecule, tissue targeted fluorophores.

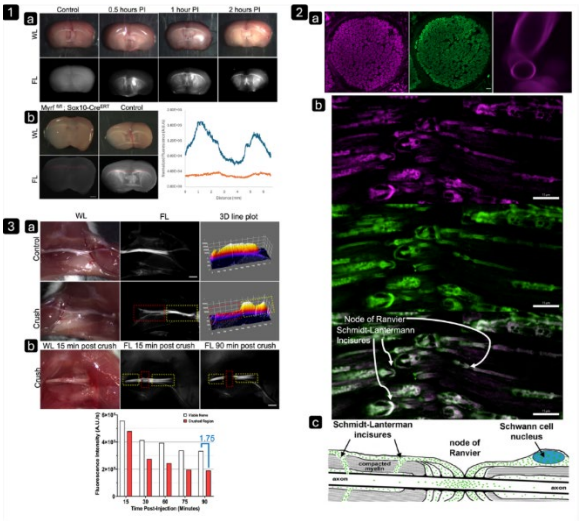


Figure caption

(1) **Demonstration of central nervous system (CNS) myelin specificity.** (a) Representative white light (WL) and fluorescence (FL) images for lead CNS fluorophore showing peak contrast within 2 hours of systemic tail vein injection in mice. (b) Representative white light (WL) and fluorescence (FL) images of lead CNS fluorophore from (a) CNS-wide myelin knockout (KO) model. Two-dimensional (2D) line plot demonstrates ablated corpus callosum white matter signal compared with wild-type (WT) control mouse and resulting fluorescence signal attenuation.

(2) Demonstration of peripheral nervous system (PNS) myelin specificity. (a) High resolution fluorescence imaging of lead 800 nm PNS-specific fluorophore (pink) and gold standard FluoroMyelin (green) exhibiting comparable staining pattern on murine brachial plexus in cross section, alongside teased nerve fibers with same fluorophore visualized by confocal microscopy, highlighting non-axonal staining. (b) Confocal fluorescence images on longitudinal teased nerve fibers as compared with schematic of Schmidt-Lantermann incisures and nodes of Ranvier, key structural elements of peripherally myelinated nerves. (c) Schematic notating the Schmidt-Lantermann incisures and node of Ranvier.5

(3) Demonstration of nerve fluorophores as nerve integrity biomarker. (a) Representative WL and FL images for mouse with pre-induced nerve crush followed by systemic administration of lead 700 nm fluorophore. (b) Similar to (a) with nerve injury performed following fluorophore administration. Nerve viable (yellow box) to crushed (red box) fluorescence ratios reach 1.75 within 90 minutes of crush, highlighting compatibility for integration with timely clinical needs.

References:

1. Bage T, Power DM. Iatrogenic peripheral nerve injury: a guide to management for the orthopaedic limb surgeon. EFORT Open Rev. 2021;6(8):607-17.
2. Sutton, P.A. et al. Fluorescence-guided surgery: comprehensive review. *BJS Open* 7, zrad049 (2023).
3. Wang, L.G. et al. Near-infrared nerve-binding fluorophores for buried nerve tissue imaging. *Sci Transl Med* 12 (2020).
4. Emery, B. et al. Myelin gene regulatory factor is a critical transcriptional regulator required for CNS myelination. *Cell* 138, 172-185 (2009).

5. Sotelo J, Canclini L, Kun A, Sotelo-Silveira J, Xu L, Wallrabe H, et al. Myosin-Va-Dependent Cell-To-Cell Transfer of RNA from Schwann Cells to Axons. *PloS one*. (2013).

#30 - Mimicking oxidative damage in γS-crystallin with site-specific incorporation of 5-hydroxytryptophan

Ms. Yeonseong (Catherine) Seo, University of California, Irvine
Dr. Zane G. Long, University of California, Irvine
Ms. Tsoler K. Demerdjian, University of California, Irvine
Mr. Acts A. Avenido, University of California, Irvine
Prof. Rachel W. Martin, University of California, Irvine

The human eye lens plays an essential role in vision by focusing light onto the retina. This transparent tissue consists of densely packed crystallin proteins that exhibit remarkable solubility despite minimal protein turnover. Post-translational damages that accrue over a lifetime can reduce crystallin solubility, resulting in the precipitation or phase-separation of protein aggregates. Oxidation is a common type of modification that can cause such opacification of the lens, particularly in age-related cataracts. Herein, we describe our efforts to study the oxidation of W163 in γS-crystallin, a structural protein particularly susceptible to oxidation. Motivated by our previous findings indicating the oxidation at this residue upon γ-irradiation, we propose that such covalent damage destabilizes the hydrophobic core of the C-terminal domain, compromising the overall solubility of the protein. To test this hypothesis, we incorporated a model oxidation product, 5-hydroxytryptophan (5-HTP), at residue 163 of γS-crystallin using a genetic code expansion (GCE) platform. The resulting oxidized variant was characterized using optical tools and compared to the wildtype. While the two displayed similar properties under ambient conditions, the oxidized variant showed diminished resilience against thermal and chemical stress. Notably, its aggregation was triggered at lower temperatures compared to the wildtype, and the underlying conformational changes were investigated using 1H–15N HSQC experiments. Our findings highlight the utility of GCE platforms in systematically evaluating site-specific post-translational modifications and provide invaluable insights to the molecular mechanisms of age-related cataracts, laying the groundwork for targeted interventions.