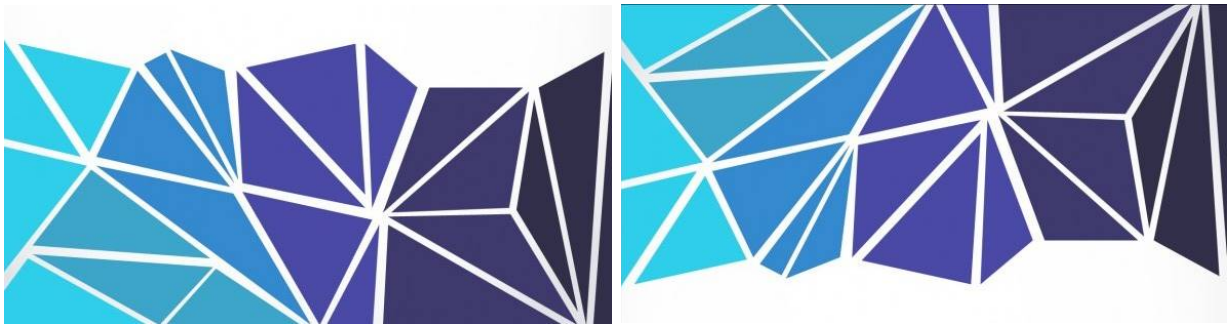
2017 Chemical Biology & Physiology Conference

Conference Program and Abstracts

December 10-13, 2017
Collaborative Life Sciences Building
2730 SW Moody Ave, Portland, OR 97201

2017 CHEMICAL BIOLOGY & PHYSIOLOGY CONFERENCE



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Sunday
December 10, 2017

11:00am-1:00pm	Registration & Welcome
1:00pm-1:10pm	**Opening remarks & announcements**
1:10pm-4:30pm	**Session 1: *Chemical Physiology*** Chair: **Martin Kelly**
1:10pm-1:45pm	Hugh Rosen
1:45pm-2:20pm	Matt Bogyo
2:20pm-2:35pm	Short Talk: Meredith Hartley
2:35pm-3:05pm	Break
3:05pm-3:40pm	Xiangshu Xiao
3:40pm-4:15pm	Jack Taunton
4:15pm-4:30pm	Short Talk: Todd Stincic
4:30pm-5:30pm	**Flash Talks**
5:30pm-7:00pm	**Poster Session with Light Dinner & Refreshments**
7:00pm-8:00pm	**Keynote Session: Craig Crews** Chair: **Kelsie Rodriguez**
8:00pm	Poster viewing

Monday
December 11, 2017

9:00am-12:35pm	**Session 2: *Signaling*** Chair: **Carsten Schultz**
9:00am-9:35am	Ulrike Eggert
9:35am-10:10am	Mike Cohen
10:10am-10:25am	Short Talk: Sebastian Hauke
10:25am-10:55am	Break
10:55am-11:30am	Dustin Maly
11:30am-12:05pm	Dorothea Fiedler
12:05pm-12:20pm	Short Talk: Stephen Shears
12:20pm-12:35pm	Short Talk: Zachary Hill
12:35pm-2:00pm	Lunch
2:00pm-4:00pm	**Session 3: *Lipids/Metabolomics*** Chair: **Ulrike Eggert**
2:00pm-2:35pm	Rami Hanoush
2:35pm-3:10pm	Mike Gelb
3:10pm-3:25pm	Short Talk: Aurélien Laguerre
3:25pm-3:40pm	Short Talk: Rob Strongin
3:40pm-4:00pm	Short Talk: Mevlut Citir
4:00pm-5:00pm	Meet the speakers & Break
5:00pm-6:00pm	**Keynote Session: Alanna Schepartz** Chair: **Jin Zhang**
6:00pm	Free evening
7:00pm	Invited Speaker Dinner

Tuesday
December 12, 2017

9:00am-12:35pm	**Session 4: *Proteins and Peptides*** Chair: **Emma Farley**
9:00am-9:35am	Ryan Mehl
9:35am-10:10am	Francis Valiyaveetil
10:10am-10:25am	Short Talk: Glenna Foight
10:25am-10:55am	Break
10:55am-11:30am	Matthew Pratt
11:30am-11:45am	Short Talk: Shahienaz Hampton
11:45am-12:20pm	Hidde Ploegh
12:20pm-12:35pm	Short Talk: Ilsa Kirby
12:35pm-1:30pm	Lunch
1:30pm-2:00pm	Meet the Editors
2:00pm-4:00pm	**Session 5: *Optical & Imaging Tools*** Chair: **Michael Cohen**
2:00pm-2:35pm	Jin Zhang
2:35pm-3:10pm	Kimberly Beatty
3:10pm-3:25pm	Short Talk: Dmytro Yushchenko
3:25pm-4:00pm	Jennifer Prescher
4:00pm-4:45pm	Meet the speakers & Break
4:45pm-5:45pm	**Keynote Session: Dirk Trauner** Chair: **Xiangshu Xiao**
6:00pm	Conference Dinner

Wednesday
December 13, 2017

9:00am-11:00pm	**Session 6: *Therapeutic Innovations*** Chair: **Tom Scanlan**
9:00am-9:35am	Tarun Kapoor
9:35am-10:10am	Heather Christofk
10:10am-10:25am	Short Talk: Bingbing Li
10:25am-11:00am	Tom Scanlan
11:00am-11:30am	Break
11:30am-12:30pm	**Keynote Session: Kevan Shokat** Chair: **Ilsa Kirby**
12:30pm-12:35pm	**Closing Remarks**
12:35pm-1:30pm	Lunch

2017 Chemical Biology & Physiology Conference

OHSU Collaborative Life Sciences Building (CLSB)
Dec. 10-13, 2017, Portland, OR

Conference Organizing Committee:
Michael Cohen, Ph.D.
Tom Scanlan, Ph.D.
Carsten Schultz, Ph.D.

Session Chairs:
Michael Cohen, Ph.D.
Ulrike Eggert, Ph.D.
Emma Farley, Ph.D. Student
Martin Kelly, Ph.D.
Ilsa Kirby, Ph.D. Student
Kelsie Rodriguez, Ph.D. Student
Tom Scanlan, Ph.D.
Carsten Schultz, Ph.D.
Xiangshu Xiao, Ph.D.
Jin Zhang, Ph.D.

Event Planners:
Amy Johnson - email: johnsamy@ohsu.edu
Elie Wiese - email: wiesee@ohsu.edu
Alanna Lapp - email: lapp@ohsu.edu

Special thanks to Jen Prissel

Conference Venue
Oregon Health & Science University
Collaborative Life Sciences Building (CLSB)
2730 S.W. Moody Avenue
Portland, OR 97201

Hotel
Hotel Modera Portland
515 SW Clay Street
Portland, OR 97201
503-308-1637

Hotel Modera to Collaborative Life Sciences Building (CLSB)

Hotel Modera
515 SW Clay Street
Portland, OR 97201

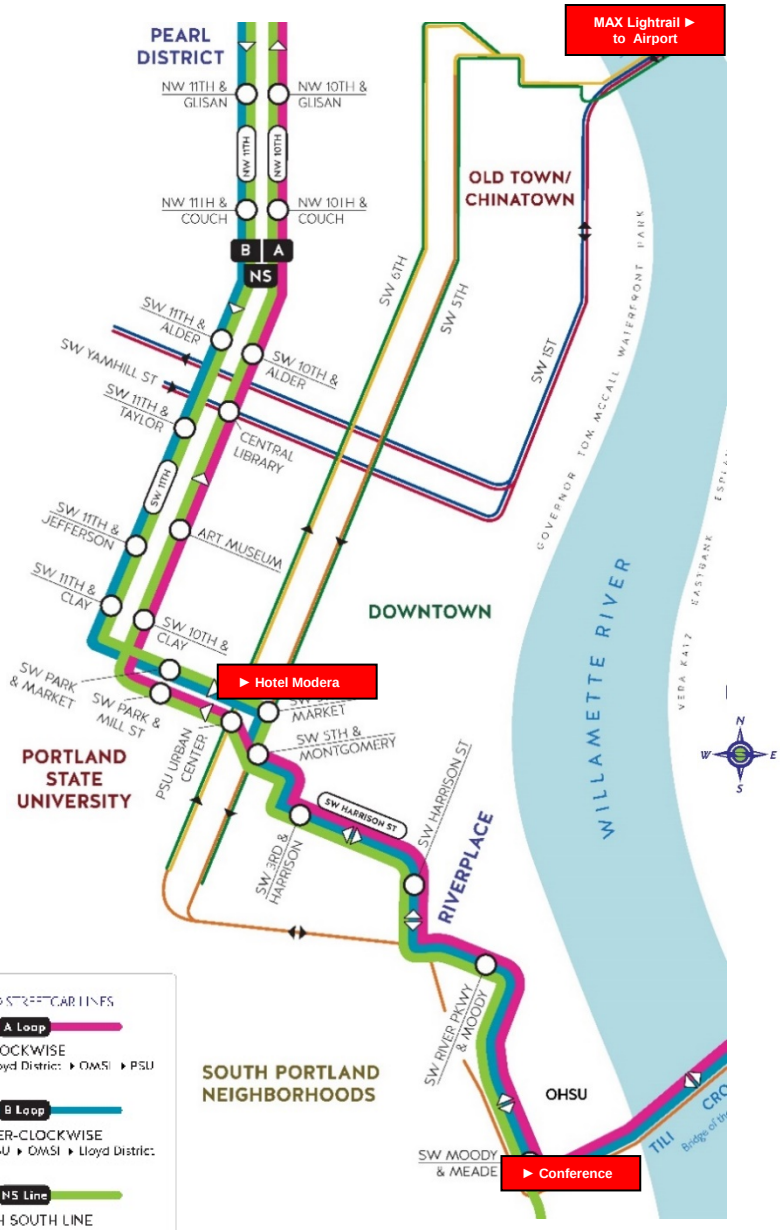
Conference Venue:
Oregon Health & Science University
Collaborative Life Sciences Building
2730 S.W. Moody Avenue
Portland, OR 97201

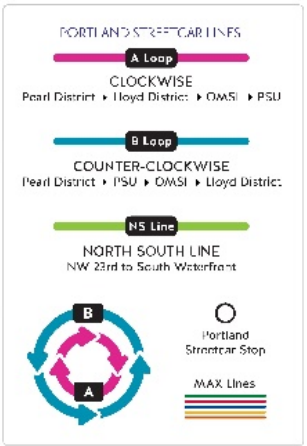
Public Transportation Directions from Hotel Modera to Conference:

- Walk one block to the SW 5th and Market streetcar stop.
- Catch the NS Line (green line) or B Loop (blue line) headed to the South Waterfront.
- Get off at SW Moody & Meade stop, in front of the Collaborative Life Sciences Building (CLSB).
- The conference is in the CLSB.

Public Transportation Directions from Conference to Hotel Modera:

- Catch the NS Line (green line) or the A Loop (pink line) at the SW Moody & Meade streetcar stop in front of CLSB headed to downtown Portland.
- Get off at PSU Urban Center stop.
- Walk 2 blocks North on SW 6th to the Hotel Modera.





Driving Directions to Collaborative Life Sciences Building (CLSB)

From the north via I-405

- Follow signs for I-405 (Salem/The Dalles).
- Take Exit 1C (S.W. 6th Ave.).
- Stay left; follow signs for 6th Ave./City Ctr./Auditorium.
- Left onto S.W. 6th Ave.
- Right onto S.W. Harrison St.
- Cross over S.W. Naito Parkway.
- Right onto S.W. Moody Ave.
- Left onto S.W. Meade St.; enter parking garage

Directions from PDX Airport via 84 W

- From NE Airport Way, take I-205 S
- Take exit 21B to I-84 W/US-30 W towards Portland
- Use the left 2 lanes to take the exit toward City Center
- Continue onto SE Morrison Bridge
- Use the right lane to take the Naito Parkway S ramp to I-5
- Merge onto SW Naito Parkway S
- Turn left onto SW Market St, which will become SW Harbor Dr
- Turn left onto SW Sheridan St
- Turn right onto SW Moody Ave
- Turn left onto S.W. Meade to enter the CLSB parking garage

From the south via I-5

- Take exit 299B, left side ("City Center/S Waterfront")
- Bear left ("City Center/S Waterfront") as exit splits
- Right onto S.W. River Parkway (first traffic signal)
- Right onto S.W. Moody Ave.
- Left onto S.W. Meade St.; enter parking garage

SUNDAY, DECEMBER 10, 2017

11:00am-1:00am	**REGISTRATION & WELCOME (CLSB Atrium)**
1:00pm-1:10pm	**OPENING REMARKS & ANNOUNCEMENTS (CLSB 1A001)**
1:10pm-4:30pm	**SESSION 1: CHEMICAL PHYSIOLOGY (CLSB 1A001)** Chair: **Martin Kelly, Ph.D.**
1:10pm-1:45pm	The Chemical Biology of Sphingosine 1-Phosphate Receptor Therapeutics. **Hugh Rosen, M.D. Ph.D.** *Professor of Molecular Medicine, The Scripps Ranch Institute*
1:45pm-2:20pm	Chemical Tools for Identification and Imaging of Hydrolases Involved in the Pathogenesis of Cancer and Infectious Diseases. **Matt Bogyo, Ph.D.** *Professor, Department of Pathology, Stanford University School of Medicine*
2:20pm-2:35pm	Short Talk: A thyroid hormone-based strategy for treating myelination disorders. **Meredith Hartley, Ph.D.** *Post-Doctoral Fellow, Physiology and Pharmacology, Oregon Health & Science University*
2:35pm-3:05pm	Break
3:05pm-3:40pm	Targeting Nuclear Lamins to Develop Novel Cancer Therapeutics. **Xiangshu Xiao, Ph.D.** *Program in Chemical Biology, Department of Physiology & Pharmacology, Knight Cancer Institute, Oregon Health & Science University*
3:40pm-4:15pm	Small-Molecule Interrogation of Protein Homeostasis. **Jack Taunton, Ph.D.** *Professor, University of California, San Francisco*
4:15pm-4:30pm	Short Talk: Estradiol modulates hypothalamic pro-opiomelanocortin neurotransmission. **Todd Stincic, Ph.D.** *Post-Doctoral Fellow, Physiology & Pharmacology, Oregon Health & Science University*
4:30pm-5:30pm	**FLASH TALKS (CLSB 1A001)**
5:30pm-7:00pm	**POSTER SESSION WITH LIGHT DINNER AND REFRESHMENTS (CLSB Atrium)**
7:00pm-8:00pm	**KEYNOTE SESSION (CLSB 1A001)** Chair: **Kelsie Rodriguez, PH.D. Student** PROTAC-induced Protein Degradation: The Chemical Equivalent of siRNA**. Craig Crews, Ph.D.** *Professor of Molecular, Cellular, and Developmental Biology; Chemistry; and Pharmacology, Yale University*
8:00pm-8:30pm	Poster viewing

MONDAY, DECEMBER 11, 2017

9:00am-12:35pm	**SESSION 2: SIGNALING (CLSB 1A001)** Chair: **Carsten Schultz, Ph.D.**
9:00am-9:35am	A Chemical Approach to Understanding Cell Division. **Ulrike Eggert**, **Ph.D.** *Professor, Department of Chemistry, King's College London*
9:35am-10:10am	Decoding Protein ADP-ribosylation Networks in Cells Using Chemical Genetic Approaches. **Michael Cohen, Ph.D.** *Associate Professor, Physiology & Pharmacology, Oregon Health & Science University*
10:10am-10:25am	Short Talk: Trace Amines are Essential Endogenous Signaling Factors for the Regulation of β-cell Activity and Insulin Secreation via TAAR1. **Sebastian Hauke, Ph.D.** *Post-Doctoral Fellow*, *European Molecular Biology Laboratory*
10:25am-10:55am	Break
10:55am-11:30am	Allosteric Modulation of Protein Kinases with ATP-Competitive Inhibitors. **Dustin J. Maly, Ph.D.** *Professor, Departments of Chemistry and Biochemistry, University of Washington, Seattle*
11:30am-12:05pm	Elucidating the Functions of Inositol Pyrophosphate Messengers with Chemical Tools. **Dorothea Fiedler, Ph.D.** *Professor, Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Berlin, Germany*
12:05pm-12:20pm	Short Talk: Structural resolution of an atypical, Cys-based protein tyrosine phosphatase reveals how it is remodeled to metabolize a polyphosphorylated cell-signal. **Stephen Shears, Ph.D.** *Senior Investigator, Signal Transduction Laboratory/ Inositol Signaling Group, National Institute of Environmental Health Sciences, National Institutes of Health*
12:20pm-12:35pm	Short Talk: Probing the Ligandable Surface of PTP1B Through Tethering-Based Ligand Scanning. **Zachary Hill, Ph.D.** *Post-Doctoral Fellow, Pharmaceutical Chemistry, University of California, San Francisco School of Pharmacy*
12:35pm-2:00pm	Lunch
2:00pm-4:00pm	**SESSION 3: LIPIDS/METABOLOMICS (CLSB 1A001)** Chair: **Ulrike Eggert, Ph.D.**
2:00pm-2:35pm	Pharmacological Modulation of Wnt Receptors in Intestinal Stem Cells. **Rami Hannoush**, **Ph.D**. *Principal Scientist, Early Discovery Biochemistry, Genentech*
2:35pm-3:10pm	Newborn Screening for Inborn Errors of Metabolism. **Michael H. Gelb, Ph.D.** *Professor and Boris and Barbara L. Weinstein Endowed Chair in Chemistry, Departments of Chemistry and Biochemistry, University of Washington, Seattle*

3:10pm-3:25pm	Short Talk: Photo-release of 2-arachidonoylglycerol in live cells: towards a new strategic approach for caged-monoacylglycerols synthesis. **Aurélien Laguerre, Ph.D.** *Post-Doctoral Fellow, Physiology & Pharmacology, Oregon Health & Science University*
3:25pm-3:40pm	Short Talk: Lysophosphatidic Acid Detection and Determination. **Rob Strongin, Ph.D.** *Professor of Organic Chemistry, Portland State University*
3:40pm-3:55pm	Short Talk: Lipidomics Analysis of Phosphoinositides and Tools for Proteomics Analysis of Phosphoinositide-Protein Interactions during Cell Cycle. **Mevlut Citir, Ph.D.** *Post-Doctoral Fellow, European Molecular Biology Laboratory*
4:00pm-5:00pm	Meet the Speakers & Break
5:00pm-6:00pm	**KEYNOTE SESSION (CLSB 1A001)** Chair: **Jin Zhang, Ph.D.** Visualizing Organelle Dynamics in Live Cells for (Almost) Forever at Super-Resolution. **Alanna Schepartz, Ph.D.** *Sterling Professor of Chemistry and Professor of Molecular, Cellular & Developmental Biology, Yale University*
6:00pm	Free Evening
7:00pm	Invited Speaker Dinner: The Picnic House 723 SW Salmon St. Portland, OR 97205 503-227-0705

TUESDAY, DECEMBER 12, 2017

9:00am-12:35pm	**SESSION 4: PROTEINS AND PEPTIDES (CLSB 1A001)** Chair: **Emma Farley, Ph.D. Student**
9:00am-9:35am	Defining Roles of NitroTyrosine in Disease Via Genetic Code Expansion. **Ryan A. Mehl, Ph.D.** *Associate Professor of Biochemistry and Biophysics, Molecular and Cellular Biology Program, Chemistry Department, & UP Facility Director of the Unnatural Protein Facility, Oregon State University*
9:35am-10:10am	Probing C-type Inactivation in K^+ channels using Unnatural Mutagenesis. **Francis I. Valiyaveetil, Ph.D.** *Associate Professor, Program in Chemical Biology, Department of Physiology & Pharmacology, Oregon Health & Science University*
10:10am-10:25am	Short Talk: Design of multi-input chemical control over cellular processes. **Glenna Foight, Ph.D.** *Post-Doctoral Fellow, Department of Chemistry, University of Washington, Seattle*
10:25am-10:55am	Break

10:55am-11:30am	Synthetic Protein Chemistry Applied to Investigate the Effects of O-GlcNAc Modification on Protein Aggregation. **Matthew Pratt, Ph.D.** *Associate Professor, Departments of Chemistry and Molecular and Computational Biology, University of Southern California*
11:30am-11:45am	Short Talk: Inhibitors of the Ras Converting Enzyme, Rce1 Disrupts RAS Localisation In Human Cells. **Shahienaz Hampton, Ph.D.** *Post-Doctoral Fellow, Division of Biological Chemistry and Drug Discovery, New York University, Abu Dhabi*
11:45am-12:20pm	Tracking Immune Responses Non-invasively by Positron Emission Tomography Using Small Antibody Fragments (VHHs or nanobodies) as Imaging Agents. **Hidde L. Ploegh, Ph.D.** *Senior Investigator, Program in Cellular and Molecular Medicine, Boston Children's Hospital and Harvard Medical School*
12:20pm-12:35pm	Short Talk: Towards a potent and selective PARP11 inhibitor. **Ilsa Kirby, Ph.D. Student** *Physiology & Pharmacology, Oregon Health & Science University*
12:35pm-1:30pm	Lunch
1:30pm-2:00pm	Meet the Editors (CLSB Second Floor)
2:00pm-4:00pm	**SESSION 5: OPTICAL & IMAGING TOOLS (CLSB 1A001)** Chair: **Michael Cohen, Ph.D.**
2:00pm-2:35pm	Illuminating the Biochemical Activity Architecture of the Cell. **Jin Zhang, Ph.D.** *Professor, University of California, San Diego*
2:35pm-3:10pm	A New Technology for Tracking Cellular Proteins at High Resolution. **Kimberly Beatty, Ph.D.** *Assistant Professor, Biomedical Engineering and Physiology and Pharmacology, Oregon Health & Science University*
3:10pm-3:25pm	Short Talk: Fluorescent photocages for spatio-temporal release of signaling lipids in cells. **Dmytro Yushchenko, Ph.D.** *Research Scientist, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of Czech Republic*
3:25pm-4:00pm	Spying on Cellular Communication with Chemical Probes. **Jennifer A. Prescher, Ph.D.** *Associate Professor, Department of Chemistry, Molecular Biology & Biochemistry, and Pharmaceutical Sciences, University of California, Irvine*
4:00pm-4:45pm	Meet the Speakers & Break
4:45pm-5:45pm	**KEYNOTE SESSION (CLSB 1A001)** Chair: **Xiangshu Xiao, Ph.D.** Controlling Lipids and GPCRs with Synthetic Photoswitches. **Dirk Trauner, Ph.D.** *Professor of Chemistry, New York University*
6:00pm	Conference Dinner (CLSB 3A001)

WEDNESDAY, DECEMBER 13, 2017

9:00am-11:00am	**SESSION 6: THERAPEUTIC INNOVATIONS (CLSB 1A001)** Chair: **Tom Scanlan, Ph.D.**
9:00am-9:35am	Leveraging Resistance for Chemical Inhibitor Design, Validation and Use. **Tarun M. Kapoor, Ph.D**. *PELS Family Professor, Biochemistry, Biophysics, Chemical Biology, and Structural Biology, The Rockefeller University*
9:35am-10:10am	Metabolic Regulation of Cell State. **Heather Christofk, Ph.D.** *Associate Professor, Biological Chemistry, Molecular & Medical Pharmacology, University of California, Los Angeles*
10:10am-10:25am	Short Talk: Targeting EWS-ATF1 in clear cell sarcoma of soft tissue (CCSST). **Bingbing Li, M.D., Ph.D.** *Senior Research Associate, Physiology & Pharmacology, Oregon Health & Science University*
10:25am-11:00am	Selective Thyroid Hormone Agonists for Central Nervous System Diseases. **Tom Scanlan, Ph.D.** *Professor, Physiology & Pharmacology, Oregon Health & Science University*
11:00am-11:30am	Break
11:30am-12:30pm	**KEYNOTE SESSION (CLSB 1A001)** Chair: **Ilsa Kirby, Ph.D. Student** Chemical Tricks for Drugging the Undruggable. **Kevan Shokat, Ph.D.** *Professor, Department of Cellular and Molecular Pharmacology, University of California, San Francisco & Howard Hughes Medical Institute*
12:30pm-12:35pm	Closing Remarks
12:35pm-1:30pm	Lunch

A NEW TECHNOLOGY FOR TRACKING CELLULAR PROTEINS AT HIGH RESOLUTION

Kimberly Beatty, Ph.D. *Assistant Professor, Oregon Health & Science University*

Recent developments in fluorescence microscopy (**FM**), electron microscopy (**EM**), and correlative light and EM (**CLEM**) offer unprecedented opportunities for illuminating cellular structures at the nanoscale. It is now feasible to visualize and quantify the spatial organization of proteins and other macromolecules that enable cells to sense and respond to their environment. However, these efforts are restricted by the shortage of methods for attaching bright fluorescent or electron-dense reporters to target proteins. To overcome this barrier, we created a new protein labeling technology using Versatile Interacting Peptide (VIP) tags. These genetically-encoded tags are mediated by a heterodimeric coiled-coil interaction. Tags are small (5-7 kDa) and compatible with a variety of chemical reporters (e.g., gold, Qdots, organic dyes). The reporter can be selected to match a particular imaging application, which enhances the versatility of our approach. We used our first coiled-coil tag, heterodimeric CoilY/CoilZ, to detect fluorophore-labeled proteins in cell lysates and in cells. Labeling was rapid and highly specific. The CoilY/Z pair was bidirectional and either Coil5 or Coil6 could be used as the probe peptide for labeling a target protein. This feature enabled us to obtain four-color images of ZipY-mCherry and ZipZ-GFP in transfected cells.
We are currently developing new VIP tags and using this technology to investigate receptor trafficking and signaling networks. Our long-term goal for this project is to empower researchers across the biological sciences to simultaneously localize and track multiple distinct proteins with nanoscale precision.

CHEMICAL TOOLS FOR IDENTIFICATION AND IMAGING OF HYDROLASES INVOLVED IN THE PATHOGENESIS OF CANCER AND INFECTIOUS DISEASES

Matt Bogyo, Ph.D. *Professor, Department of Pathology, Stanford University School of Medicine*

Hydrolases are enzymes that often play pathogenic roles in many common human diseases such as cancer, asthma, arthritis, atherosclerosis and infection by pathogens. Therefore, tools that can be used to dynamically monitor their activity can be used as diagnostic agents, as imaging contrast agents and for the identification of novel enzymes and drug leads. In this presentation, I will describe our efforts to design and synthesize small molecule probes that produce a fluorescent signal upon binding to a hydrolase target. In the first part of the presentation, I will discuss probes targeting the cysteine cathepsins and their application to real-time fluorescence guided tumor resection and other diagnostic imaging applications. In the second half of the presentation, I will present our efforts to identify novel hydrolases in the pathogenic bacteria *Staphylococcus aureus* that could be targeted to enable both treatment and non-invasive imaging of disease progression.

METABOLIC REGULATION OF CELL STATE

Heather Christofk, Ph.D. *Associate Professor, Biological Chemistry, Molecular & Medical Pharmacology, University of California, Los Angeles*

Our research focuses on metabolic transitions in cells, which coincide with important cellular processes such as differentiation, malignant transformation, immune cell activation, and virus infection. We study what regulates metabolic transitions in cells and how the cellular metabolic state influences cellular identity and function. We have recently found that pyruvate fate impacts the differentiation state of hair follicle stem cells. Hair follicle stem cells cycle between quiescence and activation during the hair cycle and give rise to all of the cells within the hair follicle. We found that genetic and pharmacological manipulation of pyruvate entry into the mitochondria influences whether hair follicle stem cells remain quiescent or activate to give rise to a new hair shaft. Therefore, metabolic perturbations in hair follicle stem cells can prevent or promote hair growth. We are currently developing small molecule modulators of pyruvate fate as hair growth promoting agents that work through topical administration. We are also investigating which metabolites are responsible for this cell fate decision as a result pyruvate fate manipulation.

DECODING PROTEIN ADP-RIBOSYLATION NETWORKS IN CELLS USING CHEMICAL GENETIC APPROACHES

Michael Cohen, Ph.D. *Associate Professor, Physiology and Pharmacology, Oregon Health & Science University*

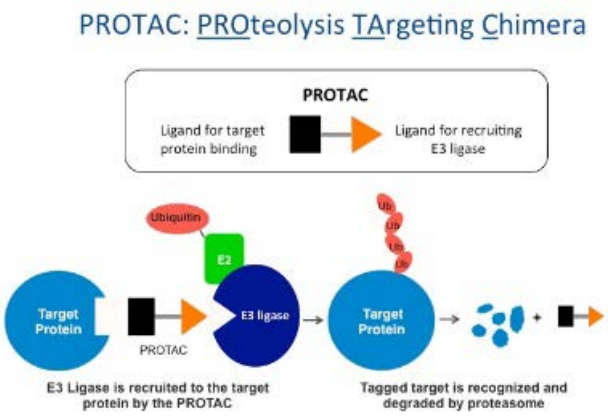
ADP-ribosylation (ADPr) is a reversible posttranslational modification that is essential for cellular function, yet little information exists regarding relevant protein substrates and target specificity. ADPr is catalyzed by a family of 17 enzymes in humans known as poly-ADP-ribose-polymerases (PARP1-16 in humans; also known as ARTDs), which transfer the ADP-ribose moiety from nicotinamide adenine dinucleotide (NAD^+) to amino acids on target proteins. The PARP family is sub-classified based on the ability of the individual PARP enzymes to catalyze the

transfer of a single ADP-ribose unit (mono-PARPs: PARP3, 6-8, 10-12, 14-16) or multiple ADP-ribose units (poly-PARPs: PARP1-2, 4, 5a, 5b) onto target proteins. Progress in understanding the specific role of a given PARP in cells has been severely limited by the inability to identify the direct targets for individual PARPs in a cellular context. To address this challenge, my laboratory has designed novel orthogonal NAD^+ analogue-engineered PARP pairs for the identification of direct protein targets of individual PARPs. We have successfully applied this approach toward the identification of the direct targets of the poly-PARP subfamily, and have recently extended this strategy to the mono-PARP subfamily. In this presentation I will discuss the identification of targets of PARP14, which is involved in normal immune function through the IL-4 signaling pathway and is a pro-survival factor in multiple myeloma and hepatocellular carcinoma. We identified 114 PARP14-specific protein targets, several of which are RNA regulatory proteins. This suggests that PARP14 plays a role in RNA regulation in cells.

PROTAC-INDUCED PROTEIN DEGRADATION: THE CHEMICAL EQUIVALENT OF siRNA

Craig Crews, Ph.D., *Professor of Molecular, Cellular, and Developmental Biology, Chemistry, Pharmacology, Yale University*

Enzyme inhibition has proven to be a successful paradigm for pharmaceutical development, however, it has several limitations. As an alternative, for the past 16 years, my lab has focused on developing Proteolysis Targeting Chimera (PROTAC), a new 'controlled proteolysis' technology that overcomes the limitations of the current inhibitor pharmacological paradigm. Based on an '*Event-driven*'



paradigm, PROTACs offer a novel, catalytic mechanism to irreversibly inhibit protein function, namely, the intracellular destruction of target proteins. This approach employs heterobifunctional molecules capable of recruiting target proteins to the cellular quality control machinery, thus leading to their degradation. We have demonstrated the ability to degrade a wide variety of targets (kinases, transcription factors, epigenetic readers) with PROTACs at picomolar concentrations. Moreover, the PROTAC technology has been demonstrated with multiple E3 ubiquitin ligases, included pVHL and cereblon.

A CHEMICAL APPROACH TO UNDERSTANDING CELL DIVISION

Ulrike Eggert, **Ph.D.** *Professor, Department of Chemistry, King's College London*

How cells regulate and execute cytokinesis, the final step in cell division, remain major unsolved questions in basic biology. It has been challenging to study cytokinesis by traditional methods because it is a very rapid and dynamic process that occupies only a small portion of the cell cycle. New approaches are needed to overcome these barriers to deeper understanding, one of which is to develop probes that act rapidly and with high temporal control. We are in the process of creating a toolbox of small molecules that inhibit different proteins and pathways in cytokinesis. I will focus on our work to understand how membranes and membrane trafficking participate in cytokinesis. Although it is known that membranes are needed to seal daughter cells after severing, very little is known about whether (and how) specific lipids are involved in cytokinesis. Massive membrane rearrangements occur during cell division, suggesting that lipids play specific roles. We used mass spectrometry to determine if the lipidome changes in dividing cells and at a division site (the midbody) and found that only very specific lipids with specific side chains accumulate (Atilla-Gokcumen, Muro et al., Cell 2014). In parallel, we systematically used RNAi to knock down lipid biosynthetic enzymes and identified enzymes required for division, which highly correlated with lipids accumulated in dividing cells. Having determined the nature of lipids involved in cell division and their biosynthetic enzymes, the next steps are to understand their functions. To further investigate the lipids' biological roles, we are using chemical biology and cell biology approaches.

ELUCIDATING THE FUNCTIONS OF INOSITOL PYROPHOSPHATE MESSENGERS WITH CHEMICAL TOOLS

Dorothea Fiedler, Ph.D. *Professor, Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Berlin, Germany*

Cells are able to modulate a range of processes according to the external environment and internal sources, by integrating signaling cues with their metabolic status. The diphosphoinositol polyphosphates (PP-InsPs) are a unique group of highly phosphorylated messengers known to control glucose uptake, insulin signaling, and energy homeostasis, and provide an important link between signaling and metabolic networks. It is thought that PP-InsPs exert their effects as allosteric small molecule regulators and via pyrophosphorylation of protein

substrates, but most details in PP-InsP signaling have thus far remained elusive.

Our group is taking chemical approaches to uncover the molecular mechanisms in PP-InsP signaling. We have applied non-hydrolyzable PP-InsP analogs for the affinity purification of inositol polyphosphate binding proteins. In parallel, we developed new strategies to detect protein pyrophosphorylation using an antibody-based approach, as well as mass spectrometry. These methods, combined with improved biochemical and analytical tools to probe inositol phosphate interconversion, are now providing the molecular details underlying the pleiotropic functions of PP-InsPs messengers in eukaryotic cells.

NEWBORN SCREENING FOR INBORN ERRORS OF METABOLISM

Michael H. Gelb, Ph.D. *Professor and Boris and Barbara L. Weinstein Endowed Chair in Chemistry, Departments of Chemistry and Biochemistry, University of Washington, Seattle*

We have developed technology based on tandem mass spectrometry for newborn screening of several inborn errors of metabolism, most notably lysosomal storage diseases. These assays are now applied in newborn screening programs worldwide. Newborn screening for these disorders allows diagnosis and initiation of treatment early in life, leading to better treatment outcomes. Most assays are based on measurement of enzymatic activities in dried blood spots, while others are based on biomarker quantification, all by tandem mass spectrometry.

PHARMACOLOGICAL MODULATION OF WNT RECEPTORS IN INTESTINAL STEM CELLS

Rami Hannoush, Ph.D., *Principal Scientist, Early Discovery Biochemistry, Genentech*

The Wnt signaling pathway is required for maintenance and self-renewal of stems cells. Aberrant Wnt signaling has also been linked to various types of cancer. This talk will discuss approaches for modulating the function of intestinal stem cells via targeting the Wnt pathway. It will also discuss our laboratory's recent progress on elucidating the biochemistry of Wnt protein lipidation and the molecular basis for the recognition of unsaturated fatty acids by the Wnt receptors.

LEVERAGING RESISTANCE FOR CHEMICAL INHIBITOR DESIGN, VALIDATION AND USE

Tarun M. Kapoor, Ph.D. *PELS Family Professor, Biochemistry, Biophysics, Chemical Biology, and Structural Biology, The Rockefeller University*

I will discuss how analysis of resistance, which is generally considered to be a limitation of molecularly targeted therapeutics, can be leveraged to address major challenges in chemical biology. First, analysis of chemotype-specific resistance can deconvolve a chemical inhibitor's mechanism of action in human cells and achieve 'gold standard' validation of its direct target, i.e. when a silent mutation in the target suppresses drug activity in cell-based and biochemical assays. Second, resistance can help with the use of chemical inhibitors as probes of cellular mechanisms. In particular, phenotypes due to target inhibition can be identified as those observed in wildtype cells, across a range of inhibitor concentrations, but not in matched cells with a silent resistant-conferring mutation in the target. Finally, resistance analyses can be used to design chemical inhibitors. I will highlight our recent efforts to design new inhibitors for AAA+ (ATPases associated with diverse cellular activities) proteins. Our approach involves testing selected chemical scaffolds against constructs with engineered silent mutations. These data yield a robust model that guides improvements in inhibitor potency and selectivity. This approach leads to spastazoline, an inhibitor of the microtubule-severing AAA+ protein spastin.

ALLOSTERIC MODULATION OF PROTEIN KINASES WITH ATP-COMPETITIVE INHIBITORS

Dustin J. Maly, Ph.D. *Professor, Departments of Chemistry and Biochemistry, University of Washington, Seattle*

Protein kinases are some of the most highly pursued targets for the development of new therapeutics by the pharmaceutical industry. A vast majority of potent protein kinase inhibitors act by competing with ATP to block the phosphotransferase activity of their targets. However, there is emerging evidence that ATP-competitive inhibitors can affect kinase interactions and functions in ways beyond blocking catalytic activity. While most inhibitors are able to interact with the ATP-binding clefts of kinases in an active conformation, a subset are conformation-selective, in that they only bind to their targets if conserved catalytic residues have been displaced from a catalytically competent conformation. The *bi-directional* allosteric relationship between the ATP-binding clefts and regulatory/scaffolding sites of protein kinases raises the intriguing possibility that these conformation-selective inhibitors may differentially influence non-phosphotransferase functions of kinases. We have shown that conformation-selective, ATP-competitive ligands are able to differentially modulate regulatory interactions,

scaffolding events, and catalytic activities that are distal to their binding sites in diverse protein kinases. Furthermore, we have found that conformation-selective inhibition can lead to divergent phenotypic effects in the cell. An overview of how the non-phosphotransferase functions of Src-family kinases can be allosterically modulated with ATP-competitive inhibitors will be presented. Furthermore, the molecular logic governing these allosteric effects will be described.

DEFINING ROLES OF NITROTYROSINE IN DISEASE VIA GENETIC CODE EXPANSION

Ryan A. Mehl, Ph.D. *Associate Professor of Biochemistry and Biophysics, Molecular and Cellular Biology Program, Chemistry Department, & UP Facility Director of the Unnatural Protein Facility, Oregon State University*

A role for reactive nitrogen species in aging as well as in over fifty human diseases including atherosclerosis, cancer, chronic pain, infection, neurodegeneration, and stroke has been demonstrated by using 3-nitrotyrosine (nitroTyr) as a biomarker. In these conditions, tyrosine nitration is not randomly distributed, but specific tyrosines on certain proteins are more readily modified. *We believe nitroTyr-modified proteins are key players in human disease.* The challenge using conventional biochemical and cell-based approaches has been how to determine which nitroTyr modifications are functionally significant and which are inconsequential. We show this hurdle can be overcome by using genetic code expansion technology to quantitatively and site-specifically incorporate nitroTyr in a targeted manner into recombinant proteins. Using this approach, I will present that specific nitroTyr-proteins in a given disease have altered properties that implicate them as key players in the development of pathology. Additionally, I will describe initial insights into the open questions of how nitration at specific tyrosines impacts select functions of calmodulin and Hsp90, and subsequently their role in calcium regulation and oxidative stress.

TRACKING IMMUNE RESPONSES NON-INVASIVELY BY POSITRON EMISSION TOMOGRAPHY USING SMALL ANTIBODY FRAGMENTS (VHHS OR NANOBODIES) AS IMAGING AGENTS

Hidde L. Ploegh, Ph.D. *Senior Investigator, Program in Cellular and Molecular Medicine, Boston Children's Hospital and Harvard Medical School*

Breakthroughs in immunotherapy of cancer are arriving in the clinic at an accelerated pace. Notwithstanding striking improvements in these antibody- and cell-based therapies, in many cases only a subset of patients responds. To understand better the reasons for success or failure, the ability to track immune responses non-invasively might be useful diagnostically and therapeutically. We have focused on the development of agents that detect myeloid cells and lymphocytes as a means of tracking anti-tumor immunity. We use single domain antibody fragments - VHHs or nanobodies- derived from camelid heavy chain-only antibodies and equipped with a sortase recognition motif to enable chemo-enzymatic installation of reporters of choice. The sortase reaction proceeds site-specifically with excellent yield, allowing modification of nanobodies with a wide array of payloads, including metal chelators to enable positron emission tomography, and click handles to execute reactions that would difficult if not impossible to achieve using conventional molecular biological methods. Using immuno-PET as the analytical tool, we find that it may be possible to use the distribution of CD8 T cells within the tumor micro-environment as a biomarker that predicts the response to checkpoint-blocking antibodies. The ability to visualize the distribution of a variety of markers in vivo non-invasively and to do so longitudinally opens up new possibilities to study immune responses in an intact living organism at a resolution that surpasses other imaging modalities such as IVIS-based imaging. While inferior to CT or MRI in terms of resolution, immuno-PET allows the characterization of the cell types present by virtue of the surface markers they display.

SYNTHETIC PROTEIN CHEMISTRY APPLIED TO INVESTIGATE THE EFFECTS OF O-GLCNAC MODIFICATION ON PROTEIN AGGREGATION

Matthew Pratt, Ph.D. *Associate Professor, Departments of Chemistry and Molecular and Computational Biology, University of Southern California*

The modification of proteins in the cytosol, nucleus, and mitochondria by the monosaccharide *N*-acetyl-glucosamine (O-GlcNAc) is required for development in mammals and Drosophila and is misregulated in a variety of diseases. One of the general functions of O-GlcNAcylation is to respond to changes in metabolism and cellular stress. Interestingly, several of the proteins that are known to form toxic aggregates in neurodegenerative diseases are known to be O-GlcNAc modified, suggesting that this modification may modulate this process. Here, I will present our investigation of the effects of O-GlcNAcylation on the biochemistry of α-synuclein, the major aggregating protein in Parkinson's disease and related dementias. Specifically, I will describe our use of synthetic protein chemistry to build O-GlcNAcylated α-synuclein and how these synthetic proteins have enabled us to characterize O-GlcNAc as an inhibitor of α-synuclein aggregation and the associated toxicity.

SPYING ON CELLULAR COMMUNICATION WITH CHEMICAL PROBES

Jennifer A. Prescher, Ph.D. *Associate Professor, Department of Chemistry, Molecular Biology & Biochemistry, and Pharmaceutical Sciences, University of California, Irvine*

Cell-to-cell interactions drive diverse aspects of human biology, and breakdowns in these networks can potentiate disease. The mechanisms by which cells exchange information in vivo, though, are not completely understood. The precise number of cell types involved, the timing and location of their interactions, and the long-term fates of the cells remain poorly characterized. This is due, in part, to a lack of tools for observing collections of cells in their native habitats. My group is developing novel chemical probes and noninvasive imaging strategies to "spy" on cells and decipher their communications in live organisms. Examples of these probes, along with their application to studies of cancer progression, will be discussed.

THE CHEMICAL BIOLOGY OF SPHINGOSINE 1-PHOSPHATE **RECEPTOR THERAPEUTICS**

Hugh Rosen, M.D., Ph.D. *Professor of Molecular Medicine, The Scripps Ranch Institute*

The chemical biology from initial reverse pharmacology to the discovery and development of ozanimod, the first NCE to emerge from the NIH Common Fund provide a case study in academic chemical discovery

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SELECTIVE THYROID HORMONE AGONISTS FOR CENTRAL NERVOUS SYSTEM DISEASES

Tom Scanlan, Ph.D. *Professor, Physiology & Pharmacology, Oregon Health & Science University*

There are several therapeutically beneficial effects triggered by thyroid hormone when it is present in higher than normal concentrations in the body. In the periphery these include lipid lowering, reduced adiposity, anti-fibrotic effects. In the central nervous system (CNS) thyroid hormone drives myelination in development and facilitates myelin repair in adults suggesting a potential therapeutic benefit in CNS diseases involving demyelination such as multiple sclerosis and leukodystrophies. The problem is that a systemic excess of thyroid hormone is also associated with adverse effects such as tachycardia, osteoporosis, and muscle wasting and there is no useful therapeutic index separating the beneficial and toxic effects. This effectively limits the clinical use of endogenous thyroid hormone to hormone replacement calling out a need for synthetic thyroid hormone agonists that are tissue selective compared to the natural hormone. My laboratory has created such compounds exemplified by the experimental drug sobetirome which is a clinical stage selective thyroid hormone agonist currently being studied in X-linked adrenoleukodystrophy (X-ALD). X-ALD is an inborn error of metabolism that results in brain and spinal cord demyelination and the associated neurological deficits along with adrenal gland dysfunction. We have also recently developed a sobetirome prodrug strategy that greatly increases the CNS exposure of sobetirome from a systemic peripheral dose. These new developments will be presented along with a mechanistic picture of how tissue selective thyroid hormone action is beneficial in X-ALD.

VISUALIZING ORGANELLE DYNAMICS IN LIVE CELLS FOR (ALMOST) FOREVER AT SUPER-RESOLUTION

Alanna Schepartz, Ph.D. *Sterling Professor of Chemistry and Professor of Molecular, Cellular & Developmental Biology, Yale University*

Imaging cellular structures and organelles in living cells by long-time-lapse super-resolution microscopy is extremely challenging, as it requires dense labeling, bright, photo-stable dyes, and non-toxic conditions. This lecture will describe a family of new chemical tools–high-density, environment-sensitive (HIDE) membrane probes–that enable exceptionally long-time-lapse, live-cell nanoscopy of discrete cellular structures and organelles with high spatio-temporal resolution. HIDE-enabled nanoscopy movies span tens of minutes, whereas movies obtained with analogously labeled proteins span tens of seconds. HIDE probes function in multiple organelles, do not require transfection or toxic additives, and are compatible with multiple super-resolution modalities. They have been applied to reveal 2D dynamics of the mitochondria, plasma membrane, and filopodia, and 2D and 3D dynamics of the endoplasmic reticulum, in living primary and cultured cells. HIDE probes also facilitate the acquisition of live cell, two-color, super-resolution images, greatly expanding the utility of nanoscopy to visualize the complex dynamic processes that underlie all cell biology.

CHEMICAL TRICKS FOR DRUGGING THE UNDRUGGABLE

Kevan Shokat, Ph.D. *Professor, Department of Cellular and Molecular Pharmacology, University of California, San Francisco & Howard Hughes Medical Institute*

Somatic mutations in the small GTPase K-Ras are the most common activating lesions found in human cancer, and are generally associated with poor response to standard therapies. Efforts to directly target this oncogene have faced difficulties due to its picomolar affinity for GTP/GDP and the absence of known allosteric regulatory sites. I will discuss the development of small molecules that irreversibly bind to a common oncogenic mutant, K-RasG12C. I will also discuss the development of a new class

of mTOR kinase inhibitors that overcomes resistance to existing first- and second-generation inhibitors of this target. The third-generation mTOR inhibitor exploits the unique juxtaposition of two drug-binding pockets to create a bivalent interaction that allows inhibition of these resistant mutants. These two projects highlight non-traditional chemical approaches for tackling highly validated yet difficult to drug targets in oncology.

SMALL-MOLECULE INTERROGATION OF PROTEIN HOMEOSTASIS

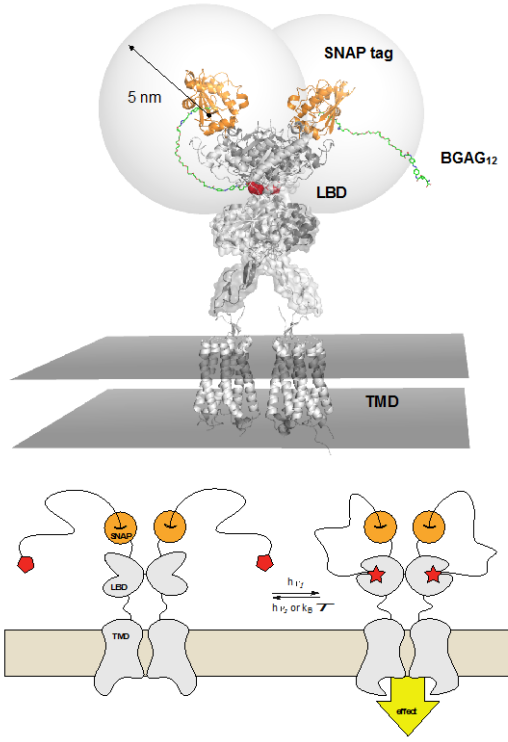
Jack Taunton, Ph.D. *Professor, University of California, San Francisco*

I will discuss the discovery of a small molecule that inhibits protein biogenesis, and at the same time, directly induces protein degradation.

CONTROLLING LIPIDS AND GPCRS WITH SYNTHETIC PHOTOSWITCHES

Dirk Trauner, Ph.D. *Professor of Chemistry, New York University*

Synthetic biology is often defined as the reshuffling of Nature's circuits on a genetic level but it could also involve the development of synthetic receptors that are sensitive to unnatural stimuli. Over the last decade, our group has



systematically explored the conversion of proteins that normally respond to small molecules, heat, membrane tension, or voltage, into photoreceptors. We usually achieve this by attaching synthetic photoswitches with modern bioconjugation techniques that work *in vivo*. Using opioid receptors and dopamine receptors (family A), GLP1 receptors (family B), and mGluRs (family C) as examples, I will show how just about any GPCR can be converted onto a photoreceptor. Several of the receptors discussed are targeted by bioactive lipids. I will show how these lipids can be reversibly activated by incorporating synthetic photoswitches, giving rise to "optolipids". We have recently synthesized a wide range of optolipids that allow for a level of temporal and spatial precision control that is otherwise difficult to reach in lipid physiology.

PROBING C-TYPE INACTIVATION IN K^+ CHANNELS USING UNNATURAL MUTAGENESIS

Francis I. Valiyaveetil, Ph.D. *Associate Professor Program in Chemical Biology, Department of Physiology & Pharmacology, Oregon Health & Science University*

K^+ channels discriminate K^+ from Na^+ in a specialized region referred to as the selectivity filter. The selectivity filter consists of four K^+ binding sites that are built using the protein backbone and the structure is highly conserved among K^+ channels. In addition to determining K^+ selectivity, the filter also participates in a gating process referred to as C-type inactivation that converts the selectivity filter from a conductive to a non-conductive state. C-type inactivation is a physiologically important process as it plays a direct role in regulating neuronal firing and in pacing cardiac action potentials. Presently, the structure of the selectivity in the C-type inactivated state or the forces that drive the selectivity filter to inactivate are not known. One of the challenges in investigating the selectivity filter is the involvement of the protein backbone and therefore the limited ability of traditional mutagenesis to precisely modify the selectivity filter. To overcome this limitation, we are using unnatural mutagenesis for modifying the selectivity filter. We have carried out unnatural mutagenesis of the selectivity filter using both synthetic and in vivo nonsense suppression approaches. Some of our recent findings on C-type inactivation will be discussed in the presentation.

TARGETING NUCLEAR LAMINS TO DEVELOP NOVEL CANCER THERAPEUTICS

Xiangshu Xiao, Ph.D. *Program in Chemical Biology, Department of Physiology & Pharmacology, Knight Cancer Institute, Oregon Health & Science University*

Nuclear lamins are type V intermediate filament (IF) proteins. In humans, three lamin genes (*LA*, *LB1* and *LB2*) encode four major lamin isoforms, lamin-A (LA), lamin-B1 (LB1), lamin-B2 (LB2) and lamin-C (LC). LA and LC are alternatively spliced products of *LA* gene. Although lamins are traditionally considered as nuclear scaffold proteins that support nuclear mechanical stability, they are also

implicated in nuclear signaling activities including the DNA double-strand breaks (DSB) repair pathways by LA. DSBs are repaired by either error-free homologous recombination (HR) or error-prone non-homologous end joining (NHEJ). However, the precise roles of LA in DSB repair remain to be elucidated. To facilitate mechanistic understanding of LA in DSB repair, we took a chemical genetics strategy to identify small molecule ligands of LA and discovered LBL1 (lamin-binding ligand 1) as the first small molecule ligand for LA. Using LBL1 as a chemical tool, we uncovered a previously unrecognized posttranslational regulation of Rad51 by LA. Our investigations show that targeting LA-Rad51 axis represents a novel strategy to develop cancer therapeutics to inhibit DSB repair.

ILLUMINATING THE BIOCHEMICAL ACTIVITY ARCHITECTURE OF THE CELL

Jin Zhang, Ph.D. *Professor, University of California, San Diego*

The complexity and specificity of many forms of signal transduction are widely suspected to require spatial microcompartmentation and dynamic modulation of the activities of signaling molecules, such as protein kinases, phosphatases and second messengers. We have developed a series of fluorescent biosensors to probe the compartmentalized signaling activities. In this talk, I will present studies where we combined genetically encoded fluorescent biosensors, superresolution imaging, mathematic modeling and targeted biochemical perturbations to probe the biochemical activity architecture of the cell.

TRACKING THE CELLULAR LOCALIZATION OF THE TRACE AMINE-ASSOCIATED RECEPTOR 1 (TAAR1) IN B-CELLS

Mireia Andreu, *European Molecular Biology Laboratory*

Sebastian Hauke, *European Molecular Biology Laboratory*

Andrey Bolbat, *European Molecular Biology Laboratory*

Carsten Schultz, *Oregon Health & Science University*

The trace amine-associated receptor 1 (TAAR1), a Gαs protein-coupled receptor, was found abundantly expressed in brain and pancreatic tissue. In accordance to this, our preliminary results indicate that trace amines (TAs) induce calcium signaling and insulin secretion from β-cells. However, it is very likely that TAAR1 activation and downstream signaling are determined by its subcellular localization, which is currently under debate in the field. To answer this question, we generated several fluorescent protein fusions and HA-tagged versions of human TAAR1 to follow cellular receptor localization and to check for redistribution and internalization events upon stimulation. In line with previous literature data, our preliminary results indicate a predominant intracellular location of hTAAR1 when overexpressed in MIN6 and HeLa cells.

However, attempts have been made to enforce cell surface expression of TAAR1 for pharmacological screens. Barack et al., 2008 reported that the addition of an asparagine-linked glycosylation site on N-terminus of TAAR1 stabilizes it at the plasma membrane. Following this, we also designed an N-glycosylated variant of hTAAR1 to monitor downstream cAMP production, calcium signaling and insulin secretion in MIN6 cells.

Apart from that, we estimate that TAAR1 activity might be also modulated by its interaction partners. Remarkably, we found that TAAR1 shows colocalization with the dopamine transporter (DAT) and dopamine receptor D3 (DRD3), when over-expressed in HeLa cells.

Taken together, the knowledge of TAAR1 subcellular localization will help to understand principles of TAAR1-signaling in β-cells and will beneficial for the design and evaluation of drug candidates to influence insulin secretion in the future.

ORIENTING PROTEINS ON SURFACES WITH SITE-SPECIFIC BIOORTHGONAL LIGATIONS

Riley Bednar, *Oregon State University*

Kelsey Kean, *Oregon State University*

Wesley Brown, *Oregon State University*

Ryan Mehl, *Oregon State University*

The functionalization of material surfaces with proteins is of great importance to a number of technologies, from industrial processes to biomedical diagnostics. However, while it has been proposed that orientation may be important to the function of such biomaterials, efforts to study such roles are hampered by a lack of rapid, quantitative, and orientation-specific immobilization techniques which will reduce non-specific fouling, and allow substoichiometric attachment of proteins onto surfaces in an orientation-controlled manner. Here, Carbonic Anydrase II (HCA)—a 30 kDA, monomeric metalloenzyme which catalyzes the interconversion of carbon dioxide to bicarbonate—is immobilized onto strained trans-cyclooctene (sTCO)-functionalized magnetic resin in an orientation-specific manner via bioorthogonal ligation with a site-specifically installed tetrazine-containing amino acid (Tet2.0).

GENETICALLY ENCODED FRET SENSOR FOR DETECTING INFECTION OF RNA VIRUSES

Andrey Bolbat, *European Molecular Biology Laboratory*

Philipp Klein, *Department of Infectious Diseases, Heidelberg University*

Alessia Ruggieri, *Department of Infectious Diseases, Heidelberg University*

Carsten Schultz, *Oregon Health & Science University*

Förster resonance energy transfer (FRET) is a quantum effect of energy transfer from donor chromophore to the acceptor one via non-radiative dipole-dipole coupling when those chromophores are positioned close enough and in the right orientation to each other. Genetically encoded FRET sensors consist of donor and acceptor fluorescent protein pair (usually CFP/YFP) and a protein based sensing domain in between which responds to the presence or the activity of a desired molecule via conformational change and, hence, resulting in a change of FRET. We developed a genetically encoded FRET sensor for detecting double stranded RNA (dsRNA) during viral infection. The sensor contains mTurquoise and cp173Venus fluorescent protein FRET pair and N-terminal domain of protein kinase R sandwiched in between.

Protein kinase R is one of the key players in antiviral response of eukaryotic cells. It consists of two domains: N-terminal dsRNA binding domain and C‑terminal catalytic domain separated by 100 amino acid non-structured region. N-terminal domain binds structure-specifically and sequence-independently dsRNA produced during replication of almost all RNA viruses which leads to activation of the kinase and eventually stalling of translation. We successfully tested the sensor in vitro, as well as in HeLa Kyoto and Huh7 cells against dsRNA showing high level of detection. The sensor also has been tested against non-infectious version of Hepatitis C Virus infection in Huh7 cells.

UNRAVELING A NEW REGULATORY MECHANISM IN SRC KINASE USING A CHEMICAL GENETICS TOOL AND SATURATION MUTAGENESIS

Sujata Chakraborty, *University of Washington*

Protein kinases are mediators of a broad variety of cellular functions. In cells, they are subjected to series of regulations that control both the specificity and magnitude of their activity. Understanding kinase structure, function and regulatory mechanism is of utmost importance since kinase misregulation can lead to numerous diseases including cancer and therefore are also the most sought-after drug targets. Here we have integrated two independent methods, Deep mutational Scanning (DMS) and a new chemical genetic approach to allosterically modulate kinase global conformation to study one of the best studied multi-domain protein kinases, Src. Src gets N-terminally myristoylated and together with a short basic SH4 domain, the N-terminal myristic acid helps in Src's membrane association in a bipartite manner. The involvement of the SH4 domain in any other regulatory mechanism is unappreciated. Combination of both of these approaches revealed that the SH4 domain acts as an intramolecular regulatory switch in Src that can be enhanced or disrupted resulting in divergent cellular outcomes. Since our integrated approach has uncovered a new layer of regulation in one of the most well characterized protein kinase, we expect them to be broadly applicable throughout the kinome to reveal new regulatory mechanisms to better understand kinase structure, function and regulations in cells.

LIPIDOMICS ANALYSIS OF PHOSPHOINOSITIDES AND TOOLS FOR PROTEOMICS ANALYSIS OF PHOSPHOINOSITIDE-PROTEIN INTERACTIONS DURING CELL CYCLE

Mevlut Citir, *European Molecular Biology Laboratory*

Rainer Mueller, *European Molecular Biology Laboratory*

Paulo Alves, *IGBMC*

Carsten Schultz, *Oregon Health & Science University*

Based on our initial findings, we showed that increased and lasting PI(3,4,5)P3 levels had different effects on interphase cells than the endogenous levels. Treatment of interphase cells with membrane-permeant PI(3,4,5)P3 compound induced changes that normally occur only in mitotic cells. Furthermore, we performed immunofluorescence assays using monoclonal antibodies against PI(3,4,5)P3 and PI(4,5)P2, and showed how endogenous PI(3,4,5)P3 and PI(4,5)P2 were localized in interphase and in mitosis. We detected significant changes in the immunodetection levels of endogenous PI(3,4,5)P3 and PI(4,5)P2 in different cell cycle phases. To find out whether the significant changes in the immunodetection levels of endogenous PI(3,4,5)P3 and PI(4,5)P2 resulted from an actual increase or decrease in their levels, we performed lipidomics analysis of phosphoinositides during cell cycle. The results suggested that the differences in the immunodetection levels were not due to an actual change but due to their differential localization in cellular compartments throughout the cell cycle, which strongly hints that PI(3,4,5)P3 and PI(4,5)P2 might have diverse binding partners in different cell cycle phases. Here, we present bifunctional derivatives of PI(3,4,5)P3 and PI(4,5)P2 for a proteomics approach to study the binding partners of PI(3,4,5)P3 and PI(4,5)P2 during cell cycle in live cells.

CAGED MORPHOLINOS AS LIGHT-DRIVEN TOOLS TO MANIPULATE GENE EXPRESSION

Davide Deodato, *New York University Abu Dhabi*

Matthew O'Connor, *New York University Abu Dhabi*

Timothy Dore, *New York University Abu Dhabi*

Morpholino oligonucleotides (MOs) represent one of the most powerful antisense reagents to study gene function in living cells as well as in developing organisms. MOs bind the complementary RNA sequence with high specificity and the presence of the phosphorodiamidate morpholino backbone gives unique properties, such as resistance to nuclease degradation, excellent water solubility, and absence of non-antisense effect. A cyclic photoactivatable version (ccMO) has been developed in our lab in order to achieve spatio-temporal control over gene silencing. In this study, the preparation and the definition of the photochemical properties of ccMOs are disclosed, together with the evaluation of the enzymatic stability against a panel of proteolytic and nucleolytic enzymes. Finally, a biological application is presented, wherein our ccMOs have been used to investigate the role of glutamic acid decarboxylase during craniofacial development in zebrafish embryos.

UTILIZATION OF AN ENGINEERED RAS RHEOSTAT LOCALIZED TO DIFFERENT RAS ISOFORMS REVEALS RAS-ERK SIGNALING DYNAMICS

Emily Dieter, *University of Washington*

John Rose, *University of Washington*

Dustin Maly, *University of Washington*

Engineered protein switches controlled through user-defined inputs are vital tools for understanding and controlling cellular dynamics. Our lab has computationally designed and developed a genetically encoded RAS rheostat that solely relies on intramolecular allosteric regulation. This system, termed Chemically Inducible Activator of RAS (CIAR), is comprised of the catalytic domain of the RAS activator Son of Sevenless (SOS), which is gated by BCL-xL-BH3 protein-peptide interactions. The BCL-xL-BH3 interaction can be disrupted using commercially available small-molecule cell-permeable inhibitors, enabling tuneable activation of endogenous RAS. Utilization of CIAR demonstrated that focal RAS activation produces distinctly different RAS/ERK signaling dynamics than those yielded through EGF activation. At present, we have generated CIAR switches with different CAAX sequences and are characterizing them through measurement of ERK phosphorylation and quantitative phosphoproteomics.

VIP TAGS: IMAGING CELLULAR PROTEINS BY FLUORESCENCE AND ELECTRON MICROSCOPY

Julia Doh, *Oregon Health & Science University*

Kimberly Beatty, *Oregon Health & Science University*

Hannah Zane, *Oregon Health & Science University*

Jonathan White, *Oregon Healthy & Science University*

Researchers use microscopy to observe proteins in their endogenous cellular environment. However, most proteins are difficult to distinguish from the rest of the cell unless they are labeled with a chemical reporter, such as a fluorescent molecule, quantum dot, or gold particle. To enable specific protein labeling by fluorescence and electron microscopy (EM), we developed Versatile Interacting Peptides (VIP) tags. VIP tags use heterodimeric coiled-coils to deliver a reporter, where one coil is genetically-encoded and the other coil, the "probe peptide," is introduced at a time choosen by the researcher (e.g., after fixation or pre-drug treatment). VIP tags are small (<8 kDa), offering a substantial size reduction from commonly used protein tags, such as the 30 kDa fluorescent proteins. VIP tags label targets specifically in a manner that is detectable by protein gel, flow cytometry, light microscopy and electron microscopy. We have thus far developed two VIP pairs, VIP Y/Z (ChemBioChem 2017) and VIP E/R (manuscript in preparation). VIP E/R enabled intracellular labeling of organelles and dynamic tracking of membrane receptors. Moreover, VIP tags enable the seamless transition between fluorescence and EM, which we demonstrated using correlative light and EM (CLEM), an advanced technique frequently limited by the scarcity of compatible tags. We plan to develop additional VIP tag pairs to be used in conjunction with each other, to allow for simultaneous imaging of multiple targets, such as the iron-sensing protein complex or HER receptors involved in breast cancer.

DEVELOPMENT OF CLICK PROBES FOR TARGET ENGAGEMENT MEASUREMENT IN SINGLE CELLS

Cécile Echalier, *European Molecular Biology Laboratory, Cellzome (a GSK company)*

Anna Rutkowska, *Cellzome (a GSK company)*

Douglas Thomson, *Cellzome (a GSK company)*

Marcel Mülbaier, *Cellzome (a GSK company)*

Giovanna Bergamini, *Cellzome (a GSK company)*

Carsten Schultz, *European Molecular Biology Laboratory*

The use of chemical probes has been shown to be a very efficient way to measure the direct binding of small molecules to endogenous targets. Recently, Cellzome developed a modular approach for visualization of drug localization, target identification and target engagement measurement in live cells (ACS Chem. Biol. 2016, 11, 2541). It is based on the bioorthogonal inverse electron demand Diels–Alder reaction between trans-cyclooctene labeled drug-based probes and tetrazine-tagged reporters (fluorophores or affinity reporters). This method was successfully applied to non-covalent inhibitors of PARP1. We are currently developing modified derivatives to optimize this methodology and increase the application scope. In particular, we employ new clickable moieties to improve the physico-chemical properties of the probes. Fluorescence-based read-outs are optimized to improve probe sensitivity and other types of read-outs are considered. Finally, experimental procedures are adapted to live cell and tissue imaging.

MECHANISTIC ANALYSIS OF THE CONTRIBUTION FROM SCAFFOLDING TO KINASE ACTIVITY IN THE WNT PATHWAY

Erin Fagnan, *University of Washington*

Maire Gavagan, *University of Washington*

Betsy Speltz, *University of Washington*

Jesse Zalatan, *University of Washington*

Scaffold proteins physically assemble multienzyme complexes and play an active role in regulating cellular decisions. In Wnt signaling, the scaffold protein Axin

tethers glycogen synthase kinase 3-beta (GSK3B) to its substrate B-catenin. This complex is required for phosphorylation of B-catenin in vivo, but how Axin contributes to the reaction at the molecular level is poorly understood. Using recombinantly expressed proteins, we reconstituted this critical step in the Wnt signaling pathway and measured B-catenin phosphorylation rates. By systematically varying the concentrations of all three components, we can define a kinetic framework for the system and quantify the contribution of the Axin scaffold to kinase activity. Adding Axin to the reaction resulted in a significant rate enhancement in the phosphorylation of B-catenin. Preliminary data indicate that Axin may cause a shift in KM, consistent with an effect on binding between GSK3B and B-catenin. This approach provides the first step to understanding how modifications to Axin during Wnt signaling regulate GSK3B activity.

A CHEMICAL-GENETIC TOOLBOX FOR ALLOSTERICALLY MODULATING KINASE CONFORMATION AND MAPPING KINASE CONFORMATION-DEPENDENT INTERACTOME IN NATIVE CELL ENVIRONMENT.

Linglan Fang, *University of Washington*

Dustin Maly, *University of Washington*

Protein kinases are structurally flexible molecules that can adopt in multiple conformations upon binding with conformation-selective ATP-competitive inhibitors and may result in distinct cellular phenotypes. We were motivated to develop three generalizable chemical-genetic tools to deconvolute the relationship between kinase conformation and functional outcomes in native cell environment, including Tool 1- Cysteine Installation for Modulating Allostery and Targeted Inhibition of Kinases (CystIMATIK), Tool 2- Co-Clickable Precipitation (Co-CP), and Tool 3- Clickable Proximity Ligation Assay (cPLA). Beyond the methodological aspects, these tools have led to the discovery of conformation-dependent localization and cell blebbing for kinase c-Src. We envision these tools would expand our understanding of conformation-controlled kinase non-catalytic functions and cellular phenotypes, and potentially make available a large resource of kinase conformation-dependent kinase-protein interactions.

FUNCTIONAL SPHINGOLIPIDS: A TOOLKIT TO INVESTIGATE SINGLE LIPID SPECIES

Emma Farley, *Oregon Health & Science University*

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Sphingolipids play critical roles in membrane structure and cell signaling. As a family, these molecules possess physical properties which convey unique rigidity and curvature upon the membranes they inhabit. As individual molecules, various sphingolipids have also been identified as participants in cellular signaling cascades ranging from apoptosis and autophagy to cell survival and immunity. Despite the importance of these molecules, it remains difficult to isolate the effects of a single lipid species. Thus, to examine the localization and protein binding partners of a single lipid, functional analogs of sphingosine as well as diacylglycerol and free fatty acids have been developed. To these molecules were added a diazirine for photocrosslinking, an alkyne for reactions with azides, and a coumarin cage to mask the probe from metabolic enzymes. Using these probes, defects in sphingosine localization and trafficking were visualized in a cellular model of Niemann-Pick Disease Type C (Höglinger et al, PNAS, 2017), a lysosomal storage disease involving defects in enzymes that metabolize sphingolipids. With these trifunctional probes as a model, the toolkit of functional lipids is being expanded to include fatty acids of varying chain length and sphingolipids with the diazirine moiety in varying locations in the molecule. These probes will be applied in particular to investigating the role of sphingolipids in the flaviviral life cycle.

ALLOSTERIC MODULATION OF THE KINASE/RNASE IRE1A BY SMALL MOLECULE AND SCAFFOLDING KINASES

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Numerous cellular perturbations can overwhelm the homeostatic capacity of the endoplasmic reticulum (ER), causing the accumulation of unfolded proteins and activation of the unfolded protein response (UPR). The UPR restores ER homeostasis (adaptive), but under prolonged stress switches to a pro-death (terminal) output. The protein kinase/RNase, IRE1α, which contains dual kinase and RNase activities, has been shown to contribute to the transition from adaptive to terminal UPR responses. During ER stress, unfolded proteins result in lumenal domain oligomerization, leading to kinase autophosphorylation and RNase activation. In cases of prolonged stress, IRE1α becomes hyperactivated leading to the endonucleolytic decay of hundreds of ER-localized mRNA, contributing to cell death. I will present mechanistic studies probing the allosteric communication between the kinase and RNase domains of IRE1α.

Inhibitors that target the ATP-binding site of IRE1α's kinase domain can have divergent allosteric effects on IRE1α's RNase activity. Some inhibitors are able to allosterically activate the RNase domain of IRE1α, while others, called KIRAs, allosterically inhibit RNase activity. We have performed biochemical and structural studies to provide insight into how ATP-competitive inhibitors can affect IRE1α oligomeric state, which directly influences RNase activity. A comprehensive structural model of how

different classes of inhibitors can divergently modulate IRE1α oligomeric state will be described. Furthermore, how different classes of inhibitors affect cell fate under ER stress will be presented.

We have also recently discovered that the tyrosine kinase Abl is capable of allosterically activating the enzymatic activities of IRE1α. Under ER stress, Abl co-localizes with IRE1α at the cytosolic face of the ER membrane, promoting IRE1α autophosphorylation and stimulating RNase activation. Mechanistic studies into how Abl serves as a scaffold for I-RE1α activation and molecular details of the IRE1α-Abl complex will be presented.

PHARMACOKINETICS AND HYPOTHALAMUS-PITUITARY-THYROID AXIS PERTURBATIONS BY A CNS TARGETING PRODRUG OF SOBETIROME

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There is currently a significant clinical need for therapeutic agents that address axonal remyelination, since the only therapies available for demyelinating disorders are limited to anti-inflammatories. Thyroid hormone is known to play an important role in promoting developmental myelination and repair, but can induce thyrotoxicosis mediated through perturbations in the hypothalamus – pituitary – thyroid neuroendocrine axis (HPT axis) at levels above those required for homeostasis. CNS permeable thyromimetic agents could offer an increased therapeutic index compared to endogenous thyroid hormone by circumventing peripheral target engagement. Sobetirome is a clinical stage thyromimetic that has been shown to have promising activity in preclinical models of demyelinating disorders like Multiple Sclerosis (MS) and X-linked adrenoleukodystrophy (X-ALD), a genetic disease that involves demyelination. Recently, we reported a successful prodrug strategy for targeting sobetirome into the CNS, while masking its exposure in the periphery. While the role of endogenous thyroid hormone within the HPT axis is well established, little is known about how thyromimetics perturb the axis. Here we report the pharmacokinetic properties of the lead CNS penetrating prodrug and how it perturbs the HPT axis in comparison to the parent drug sobetirome.

DESIGN OF MULTI-INPUT CHEMICAL CONTROL OVER CELLULAR PROCESSES

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Chemical genetic controllers of protein function are important research and therapeutic tools, but existing systems have limited ability to model the complex input-output behaviors of natural cellular processes. We describe a new chemical genetic toolset capable of responding to multiple chemical inputs to produce diverse output control modalities. Using computational design, we made two new proteins that bind specific HCV protease/inhibitor complexes, but not other inhibitors or the apo protease. Together with an existing protease inhibitor peptide, our system can produce different responses to danoprevir, grazoprevir, no inhibitor, a third HCV protease inhibitor, or combinations thereof. We demonstrate possible control architectures in applications including subcellular localization and dCas9-based transcriptional control. This new chemical genetic control paradigm enables much more complex synthetic biology control modalities for the study and manipulation of cellular processes.

SYNTHESIS OF PHOTOSWITCHABLE Δ9-TETRAHYDROCANNABINOL DERIVATIVES ENABLES OPTICAL CONTROL OF CANNABINOID RECEPTOR 1 SIGNALING

James Frank, *Massachusetts Institute of Technology*

The cannabinoid receptor 1 (CB1) is an inhibitory G protein-coupled receptor abundantly expressed in the central nervous system. It has rich pharmacology and largely accounts for the recreational use of cannabis. We describe efficient asymmetric syntheses of four photoswitchable Δ9-tetrahydrocannabinol derivatives (azo-THCs) from a central building block 3-Br-THC. Using electrophysiology and a FRET-based cAMP assay, two compounds are identified as potent CB1 agonists that change their effect upon illumination. As such, azo-THCs enable CB1-mediated optical control of inwardly-rectifying potassium channels, as well as adenylyl cyclase.

INVESTIGATING NEUTROPHIL SERINE PROTEASES ACTIVITY AT SUBCELLULAR RESOLUTION

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Cathepsin G (CG), Neutrophil Elastase (NE) and Proteinase 3 (P3) are the three neutrophil serine proteases (NSP), the eclectic players of neutrophil-mediated inflammation and pathogen fight. They are stored in primary granules and, responding to bacterial infections, they are compartmentalized inside phagolysosomes where they help digesting microorganisms. After neutrophils degranulation, they can also translocate to the cell surface, here they carry out their antimicrobial and inflammatory regulation activities. Finally, bacteria and platelets activate the secretion of DNA web-like structures able to prevent the spreading of pathogens (NETosis), and the resulting neutrophil extracellular trap (NET) is studded with NSP.

The NSP are active at these three different cellular locations, and an uncontrolled increase in their activity has been associated with the in vivo pathogenesis of lung diseases as chronic obstructive pulmonary disease (the fifth leading cause of death in the world) and cystic fibrosis (the most common lethal genetic disease affecting Caucasian population). Both conditions result in a massive neutrophilic airway inflammation promoting extensive and non-reversible tissue damaging due to uncontrolled NSP release.

Visualization and quantification of the NSP activities and interplay at subcellular resolution would shed light on their biology, it may unveil new biomarkers for disease prediction outcome and could be used for current therapies evaluation. Here, we propose the development of a new series of ratiometric FRET reporters: a lipidated CG reporter to visualize its activity on the plasma membrane, a soluble CG probe for recording the enzyme action in patients sputum supernatant an engulfable FRET beads that allow to monitor CG inside phagosomes and a NET associated probe.

RATIOMETRIC FRET REPORTERS FOR MONITORING PROTEASE ACTIVITY

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Proteases play a significant role in the breakdown of lung structure through prolonged or uncontrolled activity in different disease states, such as chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF). Monitoring these processes in a time-resolved and quantitative manner is of great interest not only in research but also in clinical applications as it enables evaluation of inhibitors and drug candidates as well as insight into protease function within complex signalling and metabolic networks.

We are developing enzyme-cleavable ratiometric FRET reporters as an effective method to quantify protease activity in intact cells and organisms. These reporters contain two fluorescent dyes covalently linked via a substrate for the enzyme of interest (either a peptide or an inhibitor-derived small molecule). Challenges in the synthesis are achieving suitable solubility for localisation of the reporter to the cellular location of the protease. In addition, maintaining protease specificity and in the case of inhibitor-derived probes introducing a cleavable bond can be problematic. [1] Here we present our latest developments.

INHIBITORS OF THE RAS CONVERTING ENZYME, RCE1 DISRUPTS RAS LOCALISATION IN HUMAN CELLS

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Inhibition of Ras maturation is considered to be a potential anticancer strategy. The Ras converting enzyme (Rce1), an integral membrane protein, mediates CaaX proteolysis, a

key modifying step required for Ras maturation and localization. There has been limited development of Rce1 inhibitors, which would greatly aid in the investigation of the physiological role of Rce1 in Ras regulation. NSC1011, a previously reported inhibitor of Rce1, identified in a medium throughput assay, was used as a starting point to develop a small library of new compounds. These exhibit moderate potency and improved selectivity against the human Rce1 (HsRce1). Moreover, these were shown to induce EGFP-Ras isoform mislocalization from the plasma membrane in a human colon carcinoma cell line. Importantly, however, several of these analogues were also shown to mislocalize EGFP-K-Ras more effectively than a known farnesyl transferase inhibitor (FTI).

A THYROID HORMONE-BASED STRATEGY FOR TREATING MYELINATION DISORDERS

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Tom Scanlan, *Oregon Health & Science University*

Thyroid hormone regulates developmental myelination by inducing the differentiation of oligodendrocyte precursor cells (OPCs) into mature, myelinating oligodendrocytes. There has been interest in the potential of exploiting thyroid hormone action in diseases that affect myelin, and thyroid hormone has been shown to promote myelin repair in animal myelin disease models including the cuprizone model of demyelination and experimental autoimmune encephalomyelitis (EAE). However, the use of thyroid hormone clinically for myelin diseases is limited by its thyrotoxic effects on cardiac, bone and muscle tissue. We are interested in characterizing thyroid hormone action in remyelination through the use of selective thyroid hormone receptor agonists, known as thyromimetics. Both thyroid hormone and thyromimetics are currently being evaluated in mouse models of multiple sclerosis and X-linked adrenoleukodystrophy. Data from these ongoing studies support the use of thyromimetics in diseases that affect CNS white matter.

TRACE AMINES ARE ESSENTIAL ENDOGENOUS SIGNALING FACTORS FOR THE REGULATION OF B-CELL ACTIVITY AND INSULIN SECRETION VIA TAAR1

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First identified in the nervous system, the trace amine associated receptor 1 (TAAR1) has come into the focus to play a broad functional role even in peripheral tissue. TAAR1 expression was recently identified in the pancreas, making it an interesting target even for the modulation of β-cell activity and insulin secretion.

In the present work, we show that increased trace amine (TA) levels (e.g. by the photolysis of a caged TA), selective TAAR1 agonists or the inhibition of TA degrading monoamine oxidases directly stimulated oscillations of cytosolic Ca2+ ([Ca2+]i oscillations) and insulin secretion from MIN6 β-cells. Opposite effects were observed when endogenous TA levels were depleted by recombinant monoamine oxidase action, by the inhibition of amino acid decarboxylase, or by the application of aromatic amine withdrawing β-cyclodextrin.

As the modulation of biochemical pathways for the synthesis and degradation of TAs immediately translated into changes of β-cell activity and insulin secretion, we inferred high metabolic turnover rates of TAs in β-cells. Selective TAAR1 antagonism shut down Ca2+ oscillations from glucose and TA-stimulated MIN6 β-cells. From all this we conclude that continuous stimulation of TAAR1 by endogenous TAs is essential for the maintenance of basal β-cell activity and insulin secretion.

PROBING THE LIGANDABLE SURFACE OF PTP1B THROUGH TETHERING-BASED LIGAND SCANNING

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Small molecules targeting allosteric sites on proteins have the potential for higher selectivity and interesting activities (e.g., modulation of substrate affinity and/or catalytic rates) compared to active-site inhibitors. Unfortunately, identifying allosteric sites on proteins, let alone developing small molecules that bind and modulate these sites, is difficult and slow. While computational and structural techniques to identify allosteric site have made progress, there still remains no general strategy to identify allosteric sites on proteins and compounds that modulate them. The Wells lab has previously demonstrated that the disulfide-fragment-based ligand discovery approach, “Tethering” can be applied to identify compounds that modulate known allosteric sites on proteins. However, identification of new allosteric sites on proteins remains a major problem. To address this, we have developed an extension of Tethering, named “Ligand Scanning”, in which we introduce individual cysteine mutants across the surface of a protein. Each of these mutants is then individually assayed for their ability to form disulfide bonds with a

library of disulfide fragments. Through this method we can identify ligandable hotspots on the protein's surface. As a proof of concept, we applied ligand scanning to identify new ligandable sites on protein-tyrosine phosphatase 1B (PTP1B). Due to its role in regulating insulin receptor kinase, PTP1B has been a long sought after drug target for the treatment of diabetes and other metabolic disorders. Unfortunately, active site inhibitors have shown poor selectivity and cell permeability. Through activity assays with the tethered complexes and crystallography, we have been able to validate a novel allosteric site on PTP1B. We believe this new site and its tethering-fragments will serve as the basis for development of new allosteric inhibitors of PTP1B.

NON-CANONICAL AMINO ACIDS FOR LIVE CELL LABELING AND FLUORESCENT SENSORS

Jan-Erik Hoffmann, *Oregon Health & Science University*

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In order to label proteins with minimal perturbations, small tags are required. Using the Amber suppression method for genetic code expansion, non-canonical amino acids can be included almost anywhere in a target protein. Possible modifications include photocrosslinkers for interaction studies and pulldown assays, as well as reactive groups for bioorthogonal click chemistry that can be specifically targeted by small organic fluorophores. With this method we were able to selectively modify the extracellular part of transmembrane receptors in living cells to study their native function. In addition we combined this method with other small molecule based labeling approaches like the FlAsH-tag to include two modifications at the same time. We created a dimerizer that can covalently and reversibly link to proteins without the need to attach additional domains and were able to make a FRET sensor for calcium based on native calmodulin. Please contact jhoffman@ohsu.edu for further questions!

CONTROLLING NANOSCALE ORGANIZATION OF THIOPHENE-BASED CONDUCTIVE POLYMERS WITH SELF-ASSEMBLING PEPTIDES

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To create nanostructured conductive polymer materials with controlled morphology and robust mechanical properties, we are exploring a bottom-up approach to polymer design. A small library of peptides containing alternating or grouped hydrophobic and hydrophilic residues was synthesized and the peptides were covalently attached to a thiophene-based monomer. Peptide sequences that could self-assemble into stable hydrogels in water were identified and the physical and mechanical properties were evaluated. Synthesis of the monomers and characterization of the gels before and after polymerization will be presented, demonstrating that this method can be exploited to create hybrid peptide-polymer materials in aqueous media.

IDENTIFICATION AND QUANTIFICATION OF ENDOGENOUS TRACE AMINES IN B-CELLS – MANIPULATING BIOCHEMICAL PATHWAYS

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The G-protein coupled receptor TAAR1 was found abundantly expressed in brain tissue, but also in the pancreas. Small aromatic primary amines, the so-called trace amines (TAs), are described to stimulate cell activity via TAAR1.

Our preliminary data suggests that TAs are synthesized and released as endogenous factors amongst β-cells within pancreatic islets, adapted to extracellular stimuli. Based on that, we turned to mass spectrometry to identify and absolutely quantify levels of endogenous TAs in the context of β-cells.

In preliminary experiments, we tested β-cell activity by monitoring Ca2+-oscillations and insulin secretion in response to the inhibition of key enzymes in β-cells. Biosynthetic pathways for TAs were already described for neuronal tissue, where TAs are generated from precursor amino acids via decarboxylation by aromatic amino acid decarboxylase (AADC) and inactivated by monoamine oxidase (MAO). Besides β-cell activity, we also directly quantified TA levels using LC-MS methods and heavy isotope standards. We find that endogenous TA levels are altered upon modulation of respective metabolic pathways in β-cells. Here, we also confirmed that TAs are produced in β-cells by the decarboxylation of precursor amino acids.

Due to high turn-over rates and their ability to penetrate live cell membranes by diffusion, it was suggested that TAs are not stored within vesicles inside cells. However, we detected elevated TA levels upon PM-depolarization. Based on these findings, we also investigated the subcellular distribution of TAs.

TOWARDS A POTENT AND SELECTIVE PARP11 INHIBITOR

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Poly-ADP-ribose polymerases (PARPs1-16) play pivotal roles in diverse cellular processes. The best-characterized PARPs (PARP1-5) catalyze poly-ADP-ribosylation (PARylation). This is due, in large part, to the availability of potent and selective inhibitors for these PARPs. There has been comparatively little success in developing selective small molecule inhibitors of PARPs (PARP6-8, 10-12, 14-16) that catalyze mono-ADP-ribosylation (MARylation), limiting our understanding of the cellular role of MARylation. We describe the structure-guided design of small molecule inhibitors of PARPs that catalyze MARylation. The most selective analog, ITK7, potently (IC₅₀ = 14 nM) inhibits the MARylation activity of PARP11. ITK7 is greater than 200-fold selective over other PARPs. ITK7 inhibits PARP11 auto-MARylation in cells in a dose-dependent manner allowing us to examine the biological mechanism of PARP11.

MINIMAL INVASIVE FLUORESCENT RECEPTOR LABELING TO STUDY EGFR INTERNALIZATION

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EGFR endocytosis has been extensively studied, often using imaging techniques involving EGFR tagged with fluorescent proteins. Although this method is a valuable tool for studying this process, advances in click chemistry and protein labeling have opened up the possibility for new methods of specifically labeling using small dyes. Genetic codon expansion allows the incorporation of non-canonical amino acids bearing ring-strained alkynes or alkenes at a specific site in a protein. The non-canonical amino acid is used to covalently bind to small fluorescent dyes via azide or tetrazine groups (1). By using hydrophilic dyes that are not able to pass the cell membrane, and by incorporating artificial amino acids in the extracellular domain of EGFR, it is possible to specifically label the portion of EGFR that is located at the plasma membrane at the time of labelling. Using this methodology, endocytosis can be observed by accumulation of the dye inside of the cell (2). Previous work in our group has shown the influence of PI(3,4,5)P3 on EGFR endocytosis. Elevated levels of PIP3 induced EGFR endocytosis and recycling in the absence of a ligand and tyrosine phosphorylation. The mechanism by which this is achieved is not known (3). To elucidate the mechanism of PIP3 induced EGFR endocytosis, we produced different EGFR mutants that were then checked for PIP3-induced internalization deficiency. For this purpose we used caged versions of PIP3 that are membrane-permeant. The caged PIP3 is not active because of a coumarin group masking the lipid head group that can be released by a flash of 405nm light. This controls the release of PIP3, both spatially and temporally, and allows us to precisely observe the effect on EGFR localization. These methods combined represent a potent platform for further studies of EGFR endocytosis that, despite years of research, is not yet fully understood.

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TWO-FOLD BIOORTHOGONAL DERIVATIZATION BY DIFFERENT FORMYLGLYCINE GENERATING ENZYMES

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For the generation of antibody-drug conjugates (ADCs) various site-specific conjugation techniques are known. One of them utilizes the formylglycine generating enzyme (FGE) which specifically oxidizes cysteine within the consensus sequence CxPxR to C^α-formylglycine (FGly). The aldehyde moiety of FGly can then be selectively addressed with different hydroxylamine or hydrazine payloads. Accordingly, this conjugation strategy enables the generation of ADCs with defined drug-antibody ratios. However, the attachment of multiple different reagents to a protein of interest with this methodology remains a challenge. In order to overcome this limitation we apply another FGly generating system. The prokaryotic AtsB, an anaerobic iron-sulfur

protein, is capable of oxidizing cysteine and serine within the recognition motif (C/S)x(A/P)xR to FGly. Hence, AtsB displays a broader substrate scope and can be sequentially used with FGE to bioorthogonally introduce two FGly residues into a polypeptide. This enables the generation of fluorescently labeled ADCs for live cell imaging experiments or the attachment of multipe different toxins.

PHOTO-RELEASE OF 2-ARACHIDONOYLGLYCEROL IN LIVE CELLS: TOWARDS A NEW STRATEGIC APPROACH FOR CAGED-MONOACYLGLYCEROLS SYNTHESIS

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Lipids participate in the functional integrity of the cell membrane, isolating the intracellular machinery from the extracellular environment while maintaining an intense active and passive trafficking from both sides of the membrane. More interestingly, individual lipid species also act as small molecular messengers and thus directly modulate cell signaling pathways. With the ambition to better understand metabolic routes regulated by lipid-protein interactions, chemical biology has already provided a well-equipped molecular toolbox in which caged lipids occupy a central place. Within the past decade, most of the attention has focused on the development of caged phospholipids, diacylglycerols and free fatty acids. So far, a successful strategy for the caging of monoacylglycerol species is still missing. Herein, we present a new strategy for the synthesis of a caged 2-arachidonoylglycerol. Effects of its photolysis are assessed monitoring oscillations of the intracellular Ca2+ concentration in live β-cells.

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

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

A SMALL MOLECULE LAMIN-BINDING LIGAND INHIBITS HOMOLOGOUS RECOMBINATION REPAIR OF DNA DOUBLE-STRAND BREAKS

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Nuclear lamins are type V intermediate filament proteins. Lamins, including LA, LB1, LB2 and LC, are the major components forming the nuclear lamina to support the mechanical stability of mammalian cell nucleus. Increasing evidence has shown that LA participates in DNA double-strand break (DSB) repair process through homologous recombination (HR). However, the mechanisms underlying this process are incompletely understood. We describe here the first small molecule ligand called **LBL1** (lamin-binding ligand 1) that directly binds LA and inhibited cancer cell growth. Further mechanistic investigations of **LBL1** reveal that LA interacts with HR recombinase Rad51 to protect Rad51 from degradation. **LBL1** inhibits LA-Rad51 interaction leading to proteasome-mediated degradation of Rad51, culminating in inhibition of HR. These results uncover a novel posttranslational regulation of Rad51 by LA and suggest that targeting the LA-Rad51 axis may represent a promising strategy to develop cancer therapeutics.

EFFECTS OF SCAFFOLDING ON JNK ACTIVATION

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The c-Jun N-terminal Kinases (JNKs), a subfamily of the Mitogen Activated Protein Kinases (MAPKs), play vital roles in important cell-fate decisions. JNKs exist in an inactive state prior to cellular stimulation, but can be activated by a variety of cellular stressors. JNK activation is achieved by phosphorylation of Thr and Tyr residues within their activation loops by their upstream MAPK Kinases. Activated JNKs are then able to phosphorylate a variety of downstream substrates in order to cause cellular change. Due to their roles in determining cell-fate, JNKs and their regulators must be tightly controlled to prevent

aberrant JNK signaling, which can lead to disease. Our current focus is on understanding the dynamics and regulation of JNK signaling modules, including how scaffolding by JNK Interacting Proteins affects the overall amplitude and specificity of JNK phosphorylation. These studies will allow us to further understand how JNKs and their regulators are able to play vital and diverse roles in many cellular decision-making processes, including both cell survival and cell death decisions.

TARGETING FATTY-ACID AMIDE HYDROLASE WITH PRODRUGS FOR CNS-SELECTIVE THERAPY

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The blood−brain barrier (BBB) can be a substantial impediment to achieving therapeutic levels of drugs in the CNS. Certain chemical functionality such as the carboxylic acid is a general liability for BBB permeability preventing significant CNS distribution of a drug from a systemic dose. Here, we report a strategy for CNS-selective distribution of the carboxylic acid containing thyromimetic sobetirome using prodrugs targeted to fatty-acid amide hydrolase (FAAH), which is expressed in the brain. A small library of amide prodrugs of sobetirome was synthesized and FAAH-catalyzed hydrolysis of the amides was examined in vitro. The most promising candidates were shown to be efficient substrates of FAAH with Vmax/KM values comparable to the natural endocannabinoid FAAH substrate anandamide. In mice, a systemic dose of sobetirome prodrug leads to a substantial ∼60-fold increase in brain distribution (Kp) of sobetirome compared to an equimolar systemic dose of the parent drug. The increased delivery of sobetirome to the brain from the prodrug was diminished by both pharmacological inhibition and genetic deletion of FAAH in vivo. The increased brain exposure of sobetirome arising from the prodrug corresponds to ∼30-fold increased potency in brain target engagement compared to the parent drug. These results suggest that FAAH-targeted prodrugs can considerably increase drug exposure to the CNS with a concomitant decrease in systemic drug levels generating a desirable distribution profile for CNS acting drugs.

OPTIMIZATION OF PEPTIDE BASED FRET-REPORTERS FOR MMP

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Matrix Metalloproteases (MMP), Neutrophil Elastase (NE) and other enzymes play a crucial role in many diseases of the lung. Usually little is known about the activity of these enzymes under disease conditions. This is mainly due to the lack of methods for assessing and quantifying the activity of relevant enzymes in clinical specimens. The aim of this work is the development of small molecule-based FRET reporters to image the proteolytic activity of MMP and NE in living cells and patient samples.

The FRET reporters are composed of a peptide sequence, which is specific for each enzyme to be monitored, and two fluorophores that flank the peptide sequence. To reveal the location of enzymatic activity, two kinds of reporters are used. Soluble reporters are designed to measure the activity in fluids by loss of FRET. Therefore the optimization of the two fluorophores, which will result in a very sensitive FRET pair, is necessary. Plasma membrane-tethered reporters are obtained by palmitoylation of the peptide sequence. These reporters function by loss of FRET and cellular internalization after the proteolytic cleavage.

FOCUSED LIBRARY OF NIR-ACTIVE CYANINE-XANTHENE HYBRIDS FOR BIOIMAGING

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Dyes that are active in the near infrared (NIR) spectral region have been of great interest in biomedical imaging. The xanthene dyes comprise some of the most common fluorophores such as fluorescein and rhodamine; however, they are not active in the NIR region. Owing to their favorable photophysical properties such as high molar absorbance, good water solubility and excellent quantum yields (ΦF= 0.95) xanthene scaffolds are among the most common labeling agents used in biological applications. Cyanine dyes are one type of the widely employed fluorophores for NIR fluorescent probe design. This work is on the synthesis of a library of cyanine-xanthene hybrid dyes with NIR optical properties for tissue and cell imaging utilizing pharmacochemical calculations predictors for inherent tissue and cell uptake. There have been few studies that have explored the systematic chemical modification for a library of fluorophores for selective uptake by specific tissue or cell types using pharmacochemical predictors. The ability to correlate

molecular structure of imaging agents and tissue-specific uptake could solve unmet clinical needs of NIR imaging agents that are inherently tissue-specific for diagnostics and therapeutic purposes.

A VERSATILE TOOLBOX FOR CREATING HETERODIMERIC KINESIN MOTORS THROUGH GENETIC CODE EXPANSION

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Kinesins are naturally occurring motor proteins that transform chemical energy stored in adenosine triphosphate (ATP) into mechanical work on hollow tube-like protein polymers known as microtubules (MTs) for various essential cellular processes. To function properly, most kinesins must coordinate the mechanochemical cycles of their two motor domains. Current approaches for generating synthetic kinesin heterodimers have greatly facilitated studies to provide insights into these coordination mechanisms but have inherent limitations making the generation of functional heterodimers challenging. Herein we provide an alternative method that relies on genetic code expansion and non-canonical amino acid (ncAA) incorporation to generate functional kinesin heterodimers. After finding that DNA oligo-based dimerization may not be suitable for kinesins, we developed an approach that relies on the orthogonal pair of ncAAs, Azido-Phenylalanine (AzF) and Tetrazine 2.0 (Tet2.0) to form functional kinesin heterodimers. This technique- the first example of an orthogonal AzF/Tet2.0-based dimerization- provides both a simple and modular alternative to current methods and has the potential to significantly expand our ability to study the fundamental principles of kinesin function.

METHANE-OXIDIZING BACTERIAL COMMUNITIES: A NOVEL SOURCE OF BIOACTIVE CHEMICAL DIVERSITY

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Most small molecule therapeutics are derived from biologically produced small molecules, and these compounds are also some of our best probes for dissecting both bacterial and mammalian cell physiology. The number of sequenced bacterial genomes has exploded in recent years, revealing a large untapped source of novel biosynthetic potential in species not traditionally relied upon for natural product discovery. One underexplored group of organisms is bacteria that grow on one carbon compounds including methanol and methane gas, which may have been overlooked in part because of their growth requirements. Through genome mining of these strains we identified a quorum sensing system in *Methylobacter tundripaludum*, a methane-oxidizing bacterium isolated from Lake Washington, Seattle, and then showed that this system activates the expression of an atypical biosynthetic gene cluster at high cell density. We subsequently isolated the product of this gene cluster and solved its structure, revealing a novel small molecule named tundrenone. We are now determining the role of this molecule in bacterial community development during growth on methane gas, and have also begun isolating additional compounds produced by these bacteria, including some with biomedical potential. This work highlights the ability to discover novel chemical diversity using bacterial species that occupy underexplored metabolic niches.

ROLE OF LAMIN B1 IN DNA DOUBLE-STRAND BREAK REPAIR

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Lamins, including lamin A, B1, B2, and C, are the major proteins of the nuclear lamina. These proteins can assemble into filamentous networks to support the mechanical stability of the nucleus. Lamins are also crucial for other nuclear metabolic processes including DNA double-strand break (DSB) repair. Misregulation of lamins has been implicated in a variety of diseases including cancer, muscular dystrophies, cardiomyopathies and premature aging. However, the exact mechanism of their involvement remains unknown. To examine the role of lamins in a more direct and concise manner we have created a novel small molecule called Lamin Binding Ligand 1 (LBL1), which binds specifically to Lamin A, C, and B1. Using LBL1 as a chemical tool, we found that lamin B1 engages DSB repair proteins and LBL1 inhibits DSB repair. These results support the potential of lamin targeting small molecules as novel cancer therapeutics.

STRUCTURAL RESOLUTION OF AN ATYPICAL, CYS-BASED PROTEIN TYROSINE PHOSPHATASE REVEALS HOW IT IS REMODELED TO METABOLIZE A POLYPHOSPHORYLATED CELL-SIGNAL

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'InsP8' is an inositol-based signaling molecule surrounded by the most densely packed 3-D array of phosphates found in Nature. Since InsP8 regulates bioenergetic homeostasis, its metabolic enzymes are potential drug targets to improve therapy for cancer, diabetes and obesity. To pursue this goal, we seek structural insights into enzymes that synthesize and metabolize InsP8. We have solved the crystal structure of an atypical, cysteine-based protein tyrosine phosphatase (siw14 from S. cerevisiae) to understand how it has been repurposed to become the most active of known InsP8 phosphatases. We describe an exceptionally electropositive P-loop that immobilizes the 5-β-phosphate for hydrolysis; the opposite end of the substrate is anchored by Lys and Arg residues in a loop that is pushed into the binding pocket due to intramolecular crowding. A canonical catalytic acid is sacrificed to avoid electrostatic clashing with the substrate, so consequently, an atypical reaction mechanism has evolved.

STABLE TRANS-CYCLOOCTENE BASED UNNATURAL AMINO ACIDS FOR IN VIVO LIGATION WITH TETRAZINES

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Rapid and specific reactivity of strained trans-cyclooctenes (TCOs) with 1,2,4,5-tetrazines in the inverse-electron demand Diels-Alder cycloaddition (IEDDAC) has been successfully utilized for modification of biomolecules with fluorophores for in vivo super-resolution microscopy. [1] Select TCO-based, strained dienophiles can react very fast with unhindered tetrazines at rates which exceed enzymatic reactions. The induced strain makes TCO-containing probes not only highly reactive but also susceptible to nucleophilic attack by thiols abundant in cells. [2] In addition, the dihydropyridazine product of the reaction between trans-cyclooct-2-en-1-ol and 1,2,4,5-tetrazine can undergo a side-elimination at a carbamate moiety with release of the linked amino acid. [3]

When carbamate derivatives of (E)-cyclooct-2-en-1-ol are used in the context of protein labelling, this side-reaction leads to heterogeneous and/or incomplete labelling with overall loss of efficiency. Therefore, there is a need in synthesis of new stable trans-cyclooctene containing amino acids. We are exploring replacement of the carbamate with various non-immolating linkers to overcome elimination problem and improve in vivo stability of the unnatural amino acids. Prepared TCO-containing amino acids are evaluated for genetic encoding in living mammalian cells. The stability of their conjugates with tetrazines is characterized by NMR and FRET studies.

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ESTRADIOL MODULATES HYPOTHALAMIC PRO-OPIOMELANOCORTIN NEUROTRANSMISSION

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Energy balance is regulated by the anorexigenic proopiomelanocortin (POMC) and orexigenic neuropeptide Y/agouti-related protein (NPY/AgRP) neurons of the hypothalamic arcuate nucleus. Although NPY/AgRP neurons are known to inhibit POMC neurons, little is known about POMC synaptic inputs to NPY/AgRP neurons. First, we employed single cell RT-PCR of $POMC^{GFP}$ neurons to determine if the machinery necessary to support GABA and glutamate release was present. We detected mRNA for vGluT2 mRNA (glutamate transporter), but not vGAT (GABA transporter). However, VMAT2 mRNA expression was documented, suggesting the presence of non-canonical GABA release. Next, we used optogenetic stimulation of POMC neurons in hypothalamic slices to examine evoked release of amino acid neurotransmitters onto NPY/AgRP neurons. $POMC^{Cre}$ mice were crossed with Ai32 mice (conditionally express eGFP and channelrhodopsin in cells expressing Cre) or injected with AAV1-Ef1α-DIO-ChR2:mCherry to produce specific expression of channelrhodopsin in POMC neurons. Female

mice were ovariectomized (OVX) 7-10 days prior to the experiments and given vehicle (oil) or 17b-estradiol (E2) injections. NPY/AgRP cells were targeted for recording based on location and electrophysiological properties. Following recording, putative NPY/AgRP neurons were harvested and identified posthoc with single cell RT-PCR. With low frequency stimulation, fast glutamatergic currents were measured in 74% (n= 62) and GABAergic currents in 26% (n= 22) of the NPY/AgRP neurons. Outward GABA currents were antagonized by GABAA receptor antagonist bicuculline and glutamatergic inward currents by AMPA and NMDA receptor antagonists CNQX and AP5, respectively. Based on a paired pulse paradigm (5 ms pulses, 50 ms interstimulus interval), the release probability of glutamate was increased in E2-treated versus oil-treated OVX female mice, as indicated by a significant decrease in the paired pulse ratio (PPR, $p<0.01$). These effects of E2 treatment were recapitulated by short term (>20 min) treatment with Gq-coupled membrane estrogen receptor agonist STX. Finally, with high frequency stimulation (20 Hz) a slow outward current developed that was antagonized by opioid receptor antagonist naloxone (1 μM, n=6), indicative that POMC neurons released β-endorphin. Therefore, E2 increases the synaptic efficacy of POMC input to NPY/AgRP neurons, thereby enhancing E2's anorexigenic effects by directly inhibiting the hunger-inducing NPY/AgRP neurons.

MIN6 CELLS STABLY EXPRESSING RINS1, A RATIOMETRIC SENSOR FOR STUDYING INSULIN SECRETION

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We have recently developed a unique probe called RINS1 for visualizing insulin secretion in response to glucose stimulation in single living -cells (Schifferer et al., A Ratiometric Sensor for Imaging Insulin Secretion in SingleCells, Cell Chemical Biology 2017). RINS1 is an attractive tool for studying the molecular mechanisms involved in insulin secretion and for screening drug candidates against diabetes. To standardize such measurement of insulin release, a MIN6 cell clone stably expressing RINS1 at a proper level was desirable. We have obtained a library of MIN6 cell lines by G418 selection in which there are many cells producing RINS1 in secretory granules with wide range of expression level. Initial characterization demonstrated that the pattern of RINS1 expression in most cells was nearly identical to experiments in cells transiently expressing RINS1. Further functional characterization and validation of single cell clones stably expressing RINS1 by spinning disc confocal and TIRF microscopy is ongoing.

FLUORESCENT PHOTOCAGES FOR SPATIO-TEMPORAL RELEASE OF SIGNALING LIPIDS IN CELLS

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Application of photocaging groups is a powerful approach to study lipid signaling. E.g. photocaging of fatty acids recently allowed us to unravel the complex nature of arachidonic acid signaling in β-cells [1]. The available repertoire of the fluorescent cages for lipid studies is, however, very poor and is limited to coumarin-based caging groups.

In this work we have created a set of new fluorescent cages based on Rhodamine fluorophores conjugated to a photolabile group [2]. We applied the new caging groups to photocage fatty acids and demonstrated that they permit visualization and quantification of caged molecules in living cells. Upon UV-irradiation, new caged lipids undergo photocleavage with the release of free fatty acid. They can be applied together with coumarin-caged lipids and permit a sequential photorelease of two signaling lipids in cells.

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The 2017 Chemical Biology and Physiology Conference is hosted by the OHSU Department of Physiology and Pharmacology. The Department of Physiology and Pharmacology emphasizes a chemical biology approach to the study of systems physiology. The distinctive mind-set of our faculty can be summed up as a desire to study cellular and molecular events in the context of the whole animal. To do this we have created a research environment in which physiologists and chemical biologists team up to create new chemical tools for probing specific signaling pathways and mechanisms in whole animal models. We believe that connecting systems physiology with cutting edge chemical biology is a powerful way to distinguish the roles of multiple inter-connected processes in vivo and is key to developing new therapeutics in the 21st century.

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