

*37th Steenbock
Symposium*

The future of
**CHEMICAL
BIOLOGY**

June 5-7, 2014

*Ebling Symposium Center
UW-Madison*

Table of Contents

Page	1	General Information
	2	Support Staff
	3	About Harry Steenbock
	3	Area Map
4 - 5		Schedule
	6	Sponsors
	7	Symposium Organizer
8 - 9		Speaker Index
	9	Chair Index
10 - 57		Speaker Bios & Abstracts
58 - 62		Poster Presenter Index
64 - 149		Poster Abstracts
151 - 155		Attendees

The 37th Steenbock Symposium *The Future of Chemical Biology*

The symposium will bring together leading scientists in the field of chemical biology to discuss their most recent research activities.

Presentations will cover topics including:

- Natural Products
- Small Molecule Modulators
- Post-translational Modification
- Imaging
- Protein Quality Control
- Chemistry for Chemical Biology

General Information

Name Badges

Symposium participants are requested to wear the name badge during all conference activities.

Registration

Pick up conference materials on Thursday, June 5th, 5:00-6:50 pm
Ebling Symposium Center Lobby, Microbial Sciences Building, 1550 Linden Drive.

Opening Reception

Thursday, June 5th, 5:00-6:50 pm. Welcoming and Keynote speakers starting at 7:00 pm.
Ebling Symposium Center Lobby, Microbial Sciences Building, 1550 Linden Drive.

Scientific Talks

All talks will be in the *Ebling Symposium Center*, Microbial Sciences Building, 1550 Linden Drive.
Talks begin at 8:55 am on Friday and conclude by 5:00 pm Saturday.

Breakfast & Lunches

Friday and Saturday. Please wear your name badge.
Registration fee includes breakfasts, breaks and lunches. See schedule for locations.

Poster Sessions

Poster Session 1: Friday 12:30-1:40 pm, *HF DeLuca Biochemistry Laboratories*, 433 Babcock Drive
Posters can go up starting at 7:00 am Friday. To be taken down at the end of the poster session.
Poster Session 2: Saturday 1:30-2:40 pm, *HF DeLuca Biochemical Sciences Building*, 440 Henry Mall
Posters can go up starting at 8:30 am Saturday. To be taken down at the end of the poster session.
Boards are numbered and reserved. Please see the reservation list in this booklet and near the boards.

Banquet

Friday, June 6th, 6:30-9:45 pm, *Wisconsin Institutes for Discovery*, 330 North Orchard Street
Banquet included in the registration fee.

The Future of Chemical Biology

37th Steenbock Symposium

University of Wisconsin – Madison

Symposium Organizer

Laura L. Kiessling

Departments of Biochemistry & Chemistry
University of Wisconsin – Madison

Symposium Coordinators

Kris Turkow

Department of Chemistry
University of Wisconsin – Madison

Leah Leighty

CALS Conference Services
University of Wisconsin – Madison

Robin Davies & Laura Vanderploeg

Symposium Web Site, Abstract Book & Illustrations

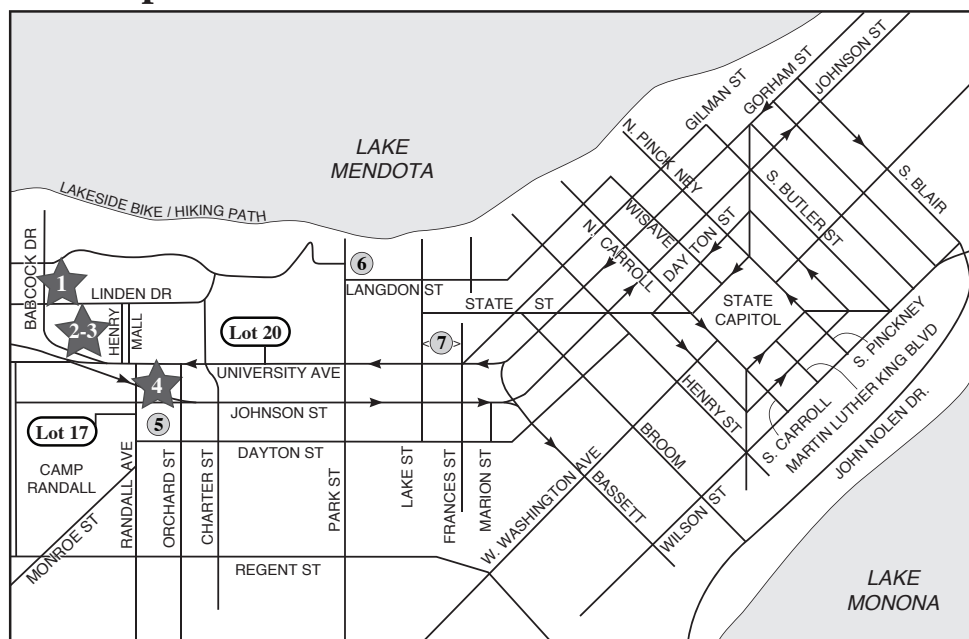
Harry Steenbock 1886–1967

A distinguished Professor of Biochemistry at the University of Wisconsin-Madison, Professor Steenbock's interests and contributions spanned many areas of nutrition and biochemistry. Of special note is his discovery of the conversion of carotenes to vitamin A, the production of vitamin D by ultraviolet irradiation, and his central participation in demonstrating that copper and iron cure nutritional anemia. The production of vitamin D by ultraviolet irradiation virtually abolished rickets, a widespread and debilitating disease. Professor Steenbock patented the irradiation process for producing vitamin D and conceived the idea of the Wisconsin Alumni Research Foundation (WARF), a nonprofit foundation which invests the proceeds from patents for support of research in the natural sciences at the University of Wisconsin-Madison. Professor Steenbock assigned his valuable patents to the Foundation, and revenue from them has provided uniquely flexible resources for fostering sustained scientific development on the Madison campus.

To honor Professor Steenbock, the Steenbock Endowment sponsors a symposium at the University of Wisconsin-Madison.



Area Map



1. Microbial Sciences Building
(Site of Symposium)
2. H.F. DeLuca Biochemistry Laboratories
(Site of Poster Session 1)
3. H.F. DeLuca Biochemical Sciences Building
(Site of Poster Session 2)
4. Wisconsin Institutes for Discovery
(Site of the Banquet)
5. Union South
6. Memorial Union
7. Lake St. & Frances St. Parking Ramps

Symposium Schedule

Thursday, June 5th, 2014

5:00 - 6:50 PM	Registration & Hors d'oeuvres <i>Ebling Symposium Center Lobby, Microbial Sciences Building, 1550 Linden Drive</i>
7:00 PM	Welcome & Opening Remarks – Laura Kiessling <i>Ebling Symposium Center</i>
7:15 - 8:15 PM	Keynote – Dennis Dougherty

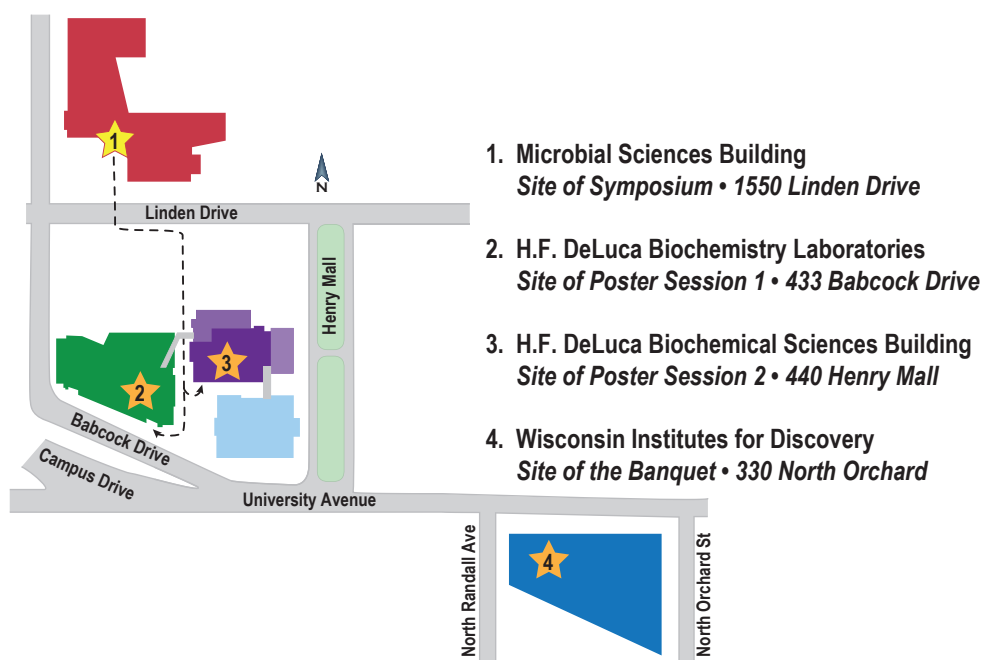
**** All talks will be in the *Ebling Symposium Center* ****
Microbial Sciences Building, 1550 Linden Drive

Friday, June 6th, 2014

8:30 - 8:50 AM	Breakfast – Ebling Lobby
8:55 - 10:30 AM	Session I • Natural Products
8:55 AM	<i>Chair:</i> Helen Blackwell
9:00 AM	Rebecca Butcher
9:30 AM	Yi Tang
10:00 AM	Frank Schroeder
10:30 - 10:50 AM	Break – Ebling Lobby
10:55 - 12:30 PM	Session II • Small Molecule Modulators
10:55 AM	<i>Chair:</i> Dehua Pei
11:00 AM	Nathanael Gray
11:30 AM	Dustin Maly
12:00 PM	Jack Taunton
12:30-1:40 PM	Lunch & Poster Session 1 <i>HF DeLuca Biochemistry Laboratories, 433 Babcock Drive</i> <i>Posters can go up starting at 7:00 am Friday. To be taken down at the end of the poster session.</i>
1:55 - 4:00 PM	Session III • Post-translational Modification
1:55 PM	<i>Chair:</i> Martha Oakley
2:00 PM	Minkui Luo
2:30 PM	Chuan He
3:00 PM	Eranthie Weerapana
3:30 PM	Mark Distefano
4:00 - 4:20	Break – Ebling Lobby
4:25 - 5:30 PM	Session IV • Imaging
4:25 PM	<i>Chair:</i> Brett Van Veller
4:30 PM	Luke Lavis
5:00 PM	Christopher Chang
6:30 PM	Reception – Wisconsin Institutes for Discovery, 330 North Orchard Street
7:30- 9:45 PM	Dinner Banquet – Wisconsin Institutes for Discovery, 330 North Orchard Street

Saturday, June 7th, 2014

8:30 - 8:50 AM	Breakfast – <i>Ebling Lobby</i>
8:55 - 11:00 AM	Session V • Protein Quality Control
8:55 AM	<i>Chair:</i> Eric Strieter
9:00 AM	Jason Gestwicki
9:30 AM	Alexander Statsyuk
10:00 AM	Peter Chien
10:30 AM	Sachdev Siddhu
11:00 - 11:20 AM	Break – <i>Ebling Lobby</i>
11:25 - 1:30 PM	Session VI • Chemistry for Chemical Biology
11:25 AM	<i>Chair:</i> Jiaoyang Jiang
11:30 AM	Ratmir Derda
12:00 PM	Nicola Pohl
12:30 PM	Geert-Jan Boons
1:00 PM	David Spiegel
1:30-2:40 PM	Lunch & Poster Session 2 <i>HF DeLuca Biochemical Sciences Building, 440 Henry Mall</i> <i>Posters can go up starting at 8:30 am Saturday. To be taken down at the end of the poster session.</i>
2:55 - 4:30 PM	Session VII • Small Molecule Modulators II
2:55 PM	<i>Chair:</i> Eric Underbakke
3:00 PM	Douglas Weibel
3:30 PM	Paul Thompson
4:00 PM	Hening Lin
4:30 PM	Closing Remarks – <i>Laura Kiessling</i>



The Steenbock Endowment

Department of Biochemistry
University of Wisconsin – Madison



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Laura L. Kiessling

Laura L. Kiessling was born in Milwaukee, WI. She received her B.S. in Chemistry from MIT and her Ph.D. in Chemistry from Yale University. After postdoctoral training in Chemical Biology at the California Institute of Technology, she returned to Wisconsin to begin her independent career at the University of Wisconsin-Madison in 1991. She currently is a Steenbock Professor of Chemistry and the Laurens Anderson Professor of Biochemistry. She also serves as the Director of the Keck Center for Chemical Genomics, the Program Director for the Chemical Biological Interface Training Program, and the Editor-In-Chief of ACS Chemical Biology. Her interdisciplinary research interests focus on elucidating and exploiting the mechanisms of cell surface recognition processes, including those that involve protein–saccharide recognition and oligosaccharide function. Another major interest of her group is multivalency and its role in recognition and signal transduction. Her research combines tools from organic synthesis, polymer chemistry, structural biology, microbiology, and molecular and cell biology to address problems in chemical biology. More information on Dr. Kiessling and her research can be found at www.biochem.wisc.edu/faculty/kiessling/lab/.

Speaker Index

Speaker	Title of Talk	Abstract Page	Day Time
Geert-Jan Boons	<i>Functional Glycomics Through Chemical Synthesis</i>	11	Saturday 12:30 PM
Rebecca A. Butcher	<i>Chemical Communication in C. elegans and other Nematodes</i>	13	Friday 9:00 AM
Christopher J. Chang	<i>Molecular Approaches to Mapping and Understanding Inorganic Chemistry in the Brain</i>	15	Friday 5:00 PM
Peter Chien	<i>Regulated Protein Degradation in Bacteria</i>	17	Saturday 10:00 AM
Ratmir Derda	<i>Discovery of Ligands from Genetically-encoded Libraries of Synthetic Molecules</i>	19	Saturday 11:30 AM
Mark Distefano	<i>Chemical Biology of Protein Prenylation</i>	21	Friday 3:30 PM
Dennis A. Dougherty	<i>Chemistry on the Brain: Understanding the Nicotine Receptor</i>	23	Thursday 7:15 PM
Jason E. Gestwicki	<i>Protein Folding in the Eye: Opportunities for Chemical Biology</i>	25	Saturday 9:00 AM
Nathanael Gray	<i>Developing New Inhibitors for Old Targets: HER3 and KRAS</i>	27	Friday 11:00 AM
Chuan He	<i>Reversible RNA Methylation in Biological Regulation</i>	29	Friday 2:30 PM
Luke D. Lavis	<i>Old Dyes, New Tricks: Building Better Fluorophores for Biology</i>	31	Friday 4:30 PM
Hening Lin	<i>Regulation of Signaling Molecules by Novel Protein Posttranslational Modifications and Sirtuins</i>	33	Saturday 4:00 PM
Minkui Luo	<i>A Journey of Developing Chemical Tools to Interrogate Protein Methyltransferases</i>	35	Friday 2:00 PM
Dustin J. Maly	<i>Allosteric Modulation of Protein Kinases with Small Molecule Inhibitors</i>	37	Friday 11:30 AM
Nicola L. B. Pohl	<i>The Challenges of Automated Oligosaccharide Synthesis for the Development of Tools for Chemical Biology</i>	39	Saturday 12:00 PM
Frank C. Schroeder	<i>Worm Language: A Natural Combinatorial Library and A Xylopyranose-based Nucleoside</i>	41	Friday 10:00 AM

Speaker Index

Speaker	Title of Talk	Abstract Page	Day Time
Sachdev S. Sidhu	<i>Modulation of Cell Signalling with Synthetic Proteins</i>	43	Saturday 10:30 AM
David A. Spiegel	<i>Using Small Molecules to Engineer and Explore Human Immunity</i>	45	Saturday 1:00 PM
Alexander V. Statsyuk	<i>The Ubiquitin System: Challenges, Opportunities, and Serendipitous Discoveries</i>	47	Saturday 9:30 AM
Yi Tang	<i>Exciting New Enzymes from Fungal Biosynthetic Pathways</i>	49	Friday 9:30 AM
Jack Taunton	<i>Selective Small-molecule Modulators of Secretory and Membrane Protein Biogenesis</i>	51	Friday 12:00 PM
Paul R. Thompson	<i>Picking the PADlock: Chemical Probes Targeting Protein Arginine Deiminase Activity</i>	53	Saturday 3:30 PM
Eranthie Weerapana	<i>Chemical-proteomic Methods to Identify Nitrosation-Sensitive Cysteines</i>	55	Friday 3:00 PM
Douglas B. Weibel	<i>Mechanical Genomics: Genome-wide Identification of Regulators of Bacterial Cell Mechanics</i>	57	Saturday 3:00 PM

Chair Index

Chair	Affiliation	Session	Day Time
Helen Blackwell	<i>University of Wisconsin-Madison</i>	<i>Session I</i>	Friday 8:55 AM
Jiaoyang Jiang	<i>University of Wisconsin-Madison</i>	<i>Session VI</i>	Saturday 11:25 AM
Laura Kiessling	<i>University of Wisconsin-Madison</i>	<i>Keynote</i>	Thursday 7:00 PM
Martha Oakley	<i>Indiana University</i>	<i>Session III</i>	Friday 1:55 PM
Dehua Pei	<i>Ohio State University</i>	<i>Session II</i>	Friday 10:55 AM
Eric Strieter	<i>University of Wisconsin-Madison</i>	<i>Session V</i>	Saturday 8:55 AM
Eric Underbakke	<i>Iowa State University</i>	<i>Session VII</i>	Saturday 2:55 PM
Brett Van Veller	<i>Iowa State University</i>	<i>Session IV</i>	Friday 4:25 PM



Geert-Jan Boons

Dr. Boons received his M.Sc. in Chemistry in 1987 and his Ph.D. in Synthetic Carbohydrate Chemistry in 1991 from the State University of Leiden in The Netherlands. Prior to joining the faculty at the CCRC in 1998, he spent seven years in the United Kingdom, first as a postdoctoral fellow at Imperial College in London and the University of Cambridge, and then as a lecturer and professor at the University of Birmingham. In 2003, Dr. Boons was awarded the Carbohydrate Research Award for Creativity in Carbohydrate Science by the European Carbohydrate Association. Also in 2003, he was elected chairman for the 2005 Gordon Research Conference on Carbohydrates. In 2004, Dr. Boons received the Horace Isbell Award by the Division of Carbohydrate Chemistry of the American Chemical Society and was appointed Franklin Professor of Chemistry in the College of Arts and Sciences at the University of Georgia. In 2012, he received the Creative Research Inventor's Award by the University of Georgia Research Foundation and was appointed UGA Foundation Distinguished Professor in Biochemical Sciences in the Franklin College of Arts and Sciences at the University of Georgia in 2013. He has been awarded by the International Carbohydrate Organization the Roy L. Whistler International Award in Carbohydrate Chemistry for 2014. Number of peer-reviewed publications: 221.

The research of the Boons group deals with the synthesis and biological functions of carbohydrates and glycoconjugates. The diversity of topics to which the group has significantly contributed includes the development of new and better methods for synthesizing exceptionally complex carbohydrates and glycoconjugates. A hallmark of Dr. Boons' research program is to employ chemical synthesized target compounds for biological and biomedical explorations and in particular, his research group has made important contributions to the understanding of immunological properties of complex oligosaccharides and glycoconjugates at the molecular level. A highlight of such program has been the design, chemical synthesis, and immunological examination of three-component vaccine candidates that offer a prospect to be employed as a therapeutic vaccine for many types of epithelial cancer.

Functional Glycomics Through Chemical Synthesis

Geert-Jan Boons

Complex Carbohydrate Research Center, University of Georgia, 315 Riverbend Road, Athens, Georgia, 30602-4712, USA. (gjboons@ccrc.uga.edu)

Despite their biological importance, there are no methods available to systematically and efficiently produce asymmetrically branched *N*-glycans needed to populate diverse glycan libraries and investigate the specificities and biology of glycan binding proteins. We report here the chemical synthesis of a pentasaccharide that is common to all eukaryotic *N*-linked glycans and is modified at positions where branching points can occur with the protecting groups levulinoyl (Lev), fluorenylmethyloxycarbonate (Fmoc), allyloxycarbonate (Alloc), and 2-naphthylmethyl (Nap). A library of complex branched bi-, tri-, and tetra-antennary structures has been generated by sequential removal of the protecting groups followed by chemical glycosylations using a diverse set of glycosyl donors. Furthermore, the use of acetylated or benzylated LacNAc and GlcNAc donors gave precursor glycans that at each antenna could be selectively extended by a panel of glycosyltransferases to rapidly give large numbers of highly complex asymmetrically substituted *N*-glycans. A similar strategy was employed to prepare a library of core-fucose modified *N*-linked glycans. The glycans were printed as microarrays and screened for binding to lectins and influenza-virus hemagglutinins, which demonstrated that recognition is modulated by presentation of minimal epitopes in the context of complex *N*-glycans.



Rebecca A. Butcher

Rebecca A. Butcher was born in Fort Myers, FL. She obtained her A.B. in chemistry from Harvard University, where she performed undergraduate research with Gregory Verdine. She then obtained her Ph.D. in chemistry and chemical biology at Harvard with Stuart Schreiber. After postdoctoral training with Jon Clardy at Harvard Medical School, Dr. Butcher returned to Florida to begin her independent career at the University of Florida in 2010. Her research interests focus on chemical communication in the model system *C. elegans*, as well as in parasitic nematodes. Her group identifies the chemical structures of nematode pheromones and studies the biosynthesis of these pheromones using a multidisciplinary approach, including RNAi-based screens, metabolomics, in vitro enzyme assays, organic synthesis, and X-ray crystallography.

Dr. Butcher's honors and awards include an NSF predoctoral fellowship, an NIH NRSA postdoctoral fellowship, and an NIH Pathway to Independence (R00) Award. She is the recipient of a New Scholar in Aging Award from the Ellison Medical Foundation and a Ralph E. Powe Junior Faculty Enhancement Award. Recently, she was named a Sloan Fellow by the Alfred P. Sloan Foundation and a Cottrell Scholar by the Research Corporation for Science Advancement. More information on Dr. Butcher and her research can be found at www.chem.ufl.edu/~butcher/.

Chemical Communication in *C. elegans* and Other Nematodes

Rebecca Butcher

The nematode *Caenorhabditis elegans* secretes the ascarosides as chemical signals to control its development and behavior. The ascarosides are structurally diverse derivatives of the 3,6-dideoxysugar ascarylose, attached to fatty acid-derived side chains. At high nematode population densities, specific ascarosides, which are together known as the dauer pheromone, trigger entry into the stress-resistant, dauer larval stage. Specific ascarosides can also influence complex behaviors, such as mating attraction and aggregation. We are investigating the biosynthetic steps that control the production of the ascarosides using a multidisciplinary approach, including RNAi-based screens, metabolomics, *in vitro* enzyme assays, organic synthesis of biosynthetic intermediates, and X-ray crystallography. The process of peroxisomal β -oxidation shortens very long-chain ascarosides to the shorter chain, dauer pheromone ascarosides in a step-wise manner (Fig. 1A). This highly regulated process is used to control the production of different pheromone components with different side chain lengths. The ascarosides can be further modified with additional groups derived from primary metabolites, such as amino acids (Fig. 1B). Our work shows that *C. elegans* co-opts pathways in primary metabolism for the biosynthesis of ascaroside secondary metabolites and thereby enables pheromone composition to change in response to environmental conditions, such as nutrient availability and temperature.

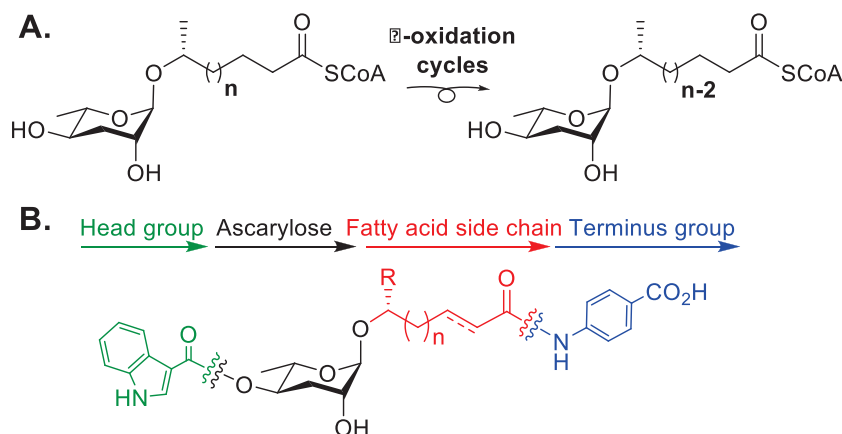


Figure 1. A. β -oxidation of ascaroside side chains. B. Modular structure of ascarosides.

Using activity-guided fractionation and NMR-based structure elucidation, we have also shown that other nematode species, including parasitic ones, use ascarosides to control their development. Many parasitic nematodes have a dauer-like infective juvenile stage, which is specialized for host infection. By characterizing the chemical signals that these nematodes use to control their development, our work will open new avenues for interfering with chemical communication in these species in order to reduce their survival.



Christopher J. Chang

Christopher J. Chang is a Professor of Chemistry and Molecular and Cell Biology and HHMI Investigator at UC Berkeley, as well as a Faculty Scientist in the Chemical Sciences Division of Lawrence Berkeley National Laboratory. He was born in Ames, IA and received his B.S. and M.S. degrees from Caltech in 1997, working with Prof. Harry Gray on spectroscopy of high-valent metal-nitrido and metal-oxo complexes. After spending a year as a Fulbright scholar in Strasbourg, France with Dr. Jean-Pierre Sauvage on chemical topology and molecular machines, Chris received his Ph.D. from MIT in 2002 under the supervision of Prof. Dan Nocera, where his graduate work focused on proton-electron transfer and oxygen catalysis. He stayed at MIT as a postdoctoral fellow with Prof. Steve Lippard, working on zinc biology and then began his independent career at UC Berkeley in Fall 2004. Research in the Chang lab is focused on chemical biology and inorganic chemistry, with particular interests in molecular imaging and catalysis applied to neuroscience, stem cells, cancer, infectious diseases, renewable energy, and green chemistry.

His group's research has been honored by awards from the Dreyfus, Beckman, Sloan, and Packard Foundations, Amgen, Astra Zeneca, and Novartis, AFAR, Technology Review, the ACS Cope Scholar Fund, and the Society for Biological Inorganic Chemistry, and in 2013 Chris was awarded the Noyce Prize at UC Berkeley for excellence in Undergraduate Teaching. Most recently Chris was awarded the 2012 ACS Eli Lilly Award in Biological Chemistry, the 2012 RSC Award in Transition Metal Chemistry, the 2013 ACS Nobel Laureate Signature Award in Graduate Education, and the 2013 Baekeland Prize. More information on the Chang lab can be found at <http://www.cchem.berkeley.edu/cjgrp/>.

Molecular Approaches to Mapping and Understanding Inorganic Chemistry in the Brain

Christopher J. Chang

Departments of Chemistry and Molecular and Cell Biology and the Howard Hughes Medical Institute, University of California, Berkeley

The brain and central nervous system requires among the highest levels of metals in the body, including transition metals like copper and iron, but misregulation of these same metal pools is a major player in aging and neurodegeneration. We are creating and applying new chemical tools to study metals and small-molecule signal/stress agents in neural systems by molecular imaging. This talk will present our latest results on developing new molecular imaging agents and their use in live cell, tissue, and animal settings.



Peter Chien

Peter Chien received B.S. degrees in Physics and Biology at MIT and his Ph.D. in Biophysics at UCSF. After postdoctoral training at MIT, he took a position at the University of Massachusetts Amherst in 2010, where he is currently an Assistant Professor in the Department of Biochemistry and Molecular Biology. The Chien lab takes an interdisciplinary approach to understand how regulated protein degradation controls fundamental biological pathways in bacteria. Most recently they have identified a novel link between proteotoxic stress and replication control, discovered new strategies for proteases to increase protein diversity through partial degradation, and reconstituted the biochemical framework for regulated protein degradation during the a bacterial cell cycle.

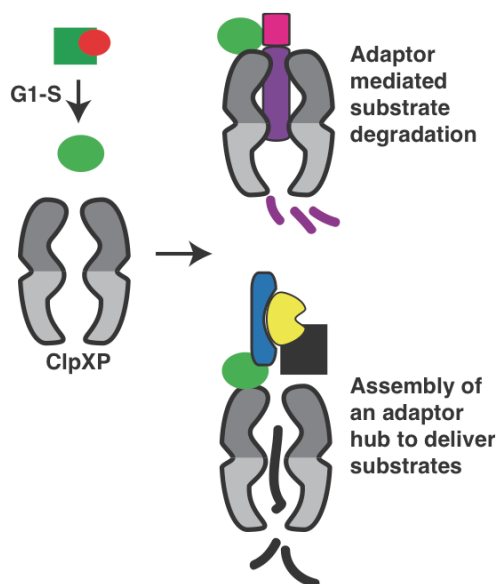
Chien's honors and awards include the Pathway to Independent Award from NIH, Jane Coffin Childs Fellowship, UCSF Krevan's Award, UCSF Clements Prize, ARCS Research Fellowship, and NSF Graduate Fellowship. More information on Dr. Chien and his group can be found at www.biochem.umass.edu/faculty/peter-chien.

Regulated Protein Degradation in Bacteria

Peter Chien

Energy dependent protein degradation ensures the complete and immediate destruction of undesired proteins with stringent selectivity. Regulated protein degradation is crucial for normal cellular growth and development as well during responses to environmental stress. In the bacteria *Caulobacter crescentus* the essential AAA+ protease ClpXP coordinates replication with differentiation via regulated proteolysis of key cell cycle factors. Using a substrate trapping approach, we have identified several new pathways that are regulated through proteolysis and revealed new features of protein degradation, such as an essential role for partial processing to generate active fragments of replication machines. Importantly, these pathways are needed for both normal growth and for the cell to respond productively to external stresses.

Although energy dependent proteases can recognize many targets directly, auxiliary factors known as adaptors can dramatically alter the substrate landscape for a given protease. These adaptors often work by tethering subsets of targets to the protease, increasing local concentration and driving degradation. During the *Caulobacter* cell cycle, regulated proteolysis of essential master regulators by ClpXP relies on several additional factors *in vivo*, but how they influence substrate choice remains poorly understood. Here, I will present our work illustrating how these additional factors alter protease selectivity by hierarchically assembling into adaptor hubs. These hubs provide for highly tuned substrate recognition by using a modular design that lends itself to the diversification of protease activity seen throughout bacteria.





Ratmir Derda

Ratmir Derda received his undergraduate degree in Physics from Moscow Institute of Physics and Technology in 2001 and Ph.D. in Chemistry from the University of Wisconsin-Madison in 2008, under the supervision of Laura L. Kiessling. From 2008 to 2011, he was a postdoctoral researcher at Harvard University working under the supervision of George M. Whitesides and Donald E. Ingber. He joined University of Alberta in 2011 as an Assistant Professor in Chemistry. In 2012, he became a principal investigator at the Alberta Glycomics Centre. Derda lab is focused on development of genetically-encoded chemical libraries, selection and evolution of bioactive ligands with dynamic properties and investigation of fundamental mechanism in cell growth and differentiation. His notable awards include Canadian Rising Star in Global Health (2011), National Academies Keck Futures Initiative Award in Synthetic Biology (2010), ACS Excellence in Graduate Polymer Science Research (2007) and Gold Medal at the XXIX International Chemistry Olympiad (1997).

Department of Chemistry and Alberta Glycomics Centre
University of Alberta, Canada.

The figure displays three chemical structures of cross-linkers used in protein chemistry, each shown in a 3D perspective view with a protein surface representation.

- Left structure:** A monofunctional cross-linker. It features a central amide bond connecting two amino acid side chains. One side chain is a primary amine (NH₂), and the other is a secondary amine (NH). The structure is labeled with 'R' for the amino acid backbone and 'B' for a specific functional group.
- Middle structure:** A bifunctional cross-linker. It consists of two identical units linked by a central azo group (-N=N-). Each unit contains a sulfonate group (-SO₃⁻) and a thiol group (-SH). The structure is labeled with 'R' for the amino acid backbone and 'S-S' for the disulfide bond.
- Right structure:** A cross-linker with a sugar moiety. It features a central amide bond connecting two amino acid side chains. One side chain is a primary amine (NH₂), and the other is a secondary amine (NH). The structure is labeled with 'R' for the amino acid backbone and 'S' for a specific functional group.

1. R Derda, S Musah, BP Orner, JR Klim, L Li, LL Kiessling “High-throughput Discovery of Synthetic Surfaces that Support Proliferation of Pluripotent Cells” *JACS*, **2010**, 132, 1289.
2. L Li, JR Klim, R Derda, AH Courtney, LL Kiessling, “Spatial control of cell fate using synthetic surfaces to potentiate TGF-beta signaling” *PNAS*, **2011**, 108, 11745.
3. S Ng, MR Jafari, W Matochko, R Derda “Quantitative Synthesis of Genetically Encoded Glycopeptide Libraries Displayed on M13 Phage” *ACS Chem. Biol.*, **2012**, 7, 1482.
4. MR Jafari, Lu Deng, S Ng, W Matochko, A Zeberof, A Elias, John S. Klassen, R Derda* "Discovery of light-responsive ligands through screening of light-responsive genetically-encoded library", *ACS Chem. Biol.*, **2014**, 9, 443.
5. F Deiss, WL Matochko, N Govindasamy, EY Lin R Derda, “Flow-Through Synthesis on Teflon-Patterned Paper To Produce Peptide Arrays for Cell-Based Assays” *Angew. Chemie Intl. Ed.*, **2014**, DOI: 10.1002/ange.201402037



Mark Distefano

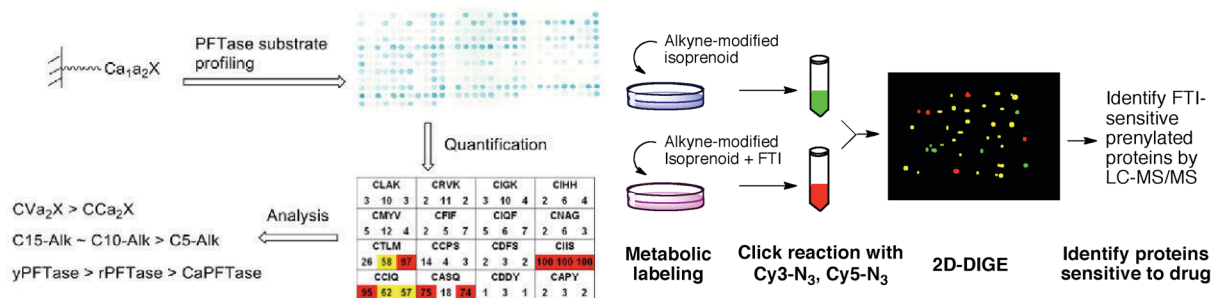
Mark Distefano was born in Baton Rouge, LA, and grew up in California and Paris, France. He received his B.A. degree in Chemistry and Biochemistry from the University of California at Berkeley in 1985 and his Ph.D. degree from Massachusetts Institute of Technology in 1989, where he worked with Professor Christopher T. Walsh. He was a Damon Runyon-Walter Winchell postdoctoral fellow in the laboratory of Peter B. Dervan at California Institute of Technology. He is currently Distinguished McKnight Professor of Chemistry and Medicinal Chemistry at the University of Minnesota and Program Director for the Chemical Biological Interface Training Program. His research is centered around the use of organic chemistry to create synthetic probes that can provide insights into biological processes. Currently, his laboratory is focused on studies of isoprenoid-utilizing enzymes, on understanding the mechanism and function of protein prenylation and on using enzymes that carry out protein lipidation for site-selective modification of enzymes for biotechnology applications.

Distefano's awards include a 3M Alumni Professorship, an American Cancer Society Junior Faculty Research Award, a National Science Foundation CAREER Award, a Pfizer Lectureship and a Merck Professorship. At Minnesota he has won the George W. Taylor/ITAS Award and the Horace T. Morse-University of Minnesota Award for Distinguished Teaching. He has been a Visiting Scientist at Genentech Corporation and served as an elected officer in several professional organizations including the American Chemical Society and the American Peptide Society. He is currently American Editor for *Bioorganic Chemistry* and a member of the Editorial Board of *Bioconjugate Chemistry*.

Chemical Biology of Protein Prenylation

Mark Distefano

Protein prenylation is a post-translational modification that consists of the addition of C₁₅ or C₂₀ isoprenoid groups to a variety of proteins. Because many proteins involved in signal transduction processes contain this modification, considerable interest exists in understanding the chemistry and biology of this phenomenon. Of particular note is the observation that protein prenylation is required for the transforming activity of mutant Ras oncoproteins; inhibition of the enzyme farnesyltransferase (which catalyzes protein prenylation) arrests the growth of transformed cells in a variety of models. A number of inhibitors of this enzyme and others in the protein prenylation pathway are currently in clinical trials for cancer therapy and other diseases.



In this presentation, experiments that report on the specificity of protein prenylation using a combination of synthetic peptide libraries and metabolic profiling will be described. First, methods for the preparation of peptide libraries containing approximately 2000 different sequences with free C-termini that can serve as substrates for protein prenylation have been developed. Screening of these libraries has revealed specificity differences between prenyltransferases from different organisms that should be useful for the design of species-specific inhibitors. In addition, a number of sequences that are efficiently prenylated have been identified that occur in the genomes of bacteria which have no endogenous prenyltransferases suggesting that these organisms hijack host enzymes to promote pathogenicity. Overall, these studies provide a useful perspective on which proteins can be prenylated. In complementary work, metabolic labeling experiments using alkyne-containing isoprenoid analogues can be used to reveal which proteins within mammalian cells are prenylated. Immunoblotting experiments in conjunction with phenotypic assays demonstrate that such analogues can be incorporated into prenylated proteins and that the resulting proteins are functional. Enrichment via biotin pull-down or fluorescent labeling (both mediated via click reactions) followed by proteomic analysis has allowed a number of prenylated proteins to be identified. Differential gel electrophoretic (DIGE) methods have facilitated the identification of specific prenylated proteins whose levels increase or decrease in response to prenylation inhibitors and may serve as improved biomarkers for clinical applications. Finally, click reaction-mediated fluorescent labeling of cells after metabolic incorporation of alkyne-functionalized isoprenoids is allowing the intracellular localization and overall levels of prenylated proteins to be studied.



Dennis A. Dougherty

Dennis A. Dougherty is the George Grant Hoag Professor of Chemistry at the California Institute of Technology. He received his BS from Bucknell University, followed by doctoral work at Princeton University with Kurt Mislow and postdoctoral study with Jerome Berson at Yale University. He is a member of the National Academy of Sciences and a fellow of the American Academy of Arts and Science and the American Association for the Advancement of Science. He has received numerous awards, including the Biopolymers Murray Goodman Award, the ACS James Flack Norris Award for Physical Organic Chemistry, the Hoffmann Medal of the University of Zurich, the Tolman Medal, the AstraZeneca Excellence in Chemistry Award, the Arthur C. Cope Scholar Award, and has been designated a Javits Neuroscience Investigator by NIH. Dougherty has been recognized with several teaching awards at Caltech, including the Richard Feynman Prize. He is also the co-author, with Professor Eric Anslyn, of the influential textbook, *Modern Physical Organic Chemistry*.

Dougherty is perhaps best known for the development of the cation- π interaction, a powerful noncovalent binding interaction that is widely employed in both small molecule and macromolecular recognition in biology and chemistry. His current research is focused on molecular neurobiology, applying the mindset and tools of physical organic chemistry to the complex proteins of neuroscience. Target receptors include the nicotinic acetylcholine receptor, the 5-HT₃ (serotonin) receptor, and voltage-gated ion channels. A highlight of this work has been the evaluation of the binding modes of nicotine at receptors in the CNS vs. the periphery, revealing the critical role that cation- π interactions play in establishing the addictive properties of nicotine.

Chemistry on the Brain: Understanding the Nicotine Receptor.

*Dennis A. Dougherty
Division of Chemistry and Chemical Engineering
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The human brain is the most complex object known to man. It presents daunting challenges at all levels, from the anatomical, to the cellular, to the molecular. Our work seeks to provide a chemical-scale understanding of the molecules of memory, thought, and sensory perception; of Alzheimer's, Parkinson's, and schizophrenia. An area of particular interest has been the chemistry of nicotine addiction. The initial chemical event of nicotine addiction involves nicotine binding to and activating acetylcholine (ACh) receptors in the brain. Using the mindset and methodologies of physical organic chemistry, we have probed these complex membrane proteins with a precision and subtlety normally associated with small molecule studies. We have established that the cation- π interaction plays a pivotal role in promoting the high potency of nicotine in the brain, leading to its addictive properties. We have also discovered key hydrogen bonding interactions that uniquely contribute to the binding of nicotine to ACh receptors. These chemical studies provide a high-precision structural model for the interaction of potent drugs at brain receptors.



Jason E. Gestwicki

Jason E. Gestwicki received his B.S. in Chemistry from SUNY Fredonia in 1997 and his Ph.D. in Biochemistry at the University of Madison-Madison in 2002. He was a Helen Hay Whitney postdoctoral fellow at Stanford University before starting his independent career at the University of Michigan in 2005. In 2013, he relocated his group to the University of California at San Francisco, where he is currently an Associate Professor in the Department of Pharmaceutical Chemistry and the Institute for Neurodegenerative Disease. His main research interests are in protein homeostasis, molecular chaperones, protein-protein interactions and protein misfolding diseases. His group's strategy is to develop new chemical probes that they use to acutely perturb the function of chaperones in disease models, revealing the underlying logic of protein quality control networks. In addition, they use these probes as leads towards the development of therapeutics for protein misfolding disorders.

Gestwicki is an elected fellow of the American Association for the Advancement of Science and a recipient of an NSF CAREER award and a McKnight Foundation Brain Disorders Award. He was named a "Young Innovator" at UCSF in 2014. He also serves on the Editorial Advisory boards of *ACS Chemical Biology*, *Journal of Medicinal Chemistry* and *Chemical Biology & Drug Design*. He has published 100+ manuscripts and 18 patents and co-founded Amplyx Pharmaceuticals.

Protein Folding in the Eye: Opportunities for Chemical Biology

Jason Gestwicki

The crystallin proteins are abundant components of the lens, where they provide that tissue's remarkable combination of transparency, strength and elasticity. AlphaB-crystallin is the major chaperone of the lens, which stabilizes the other crystallins and protects against aggregation. However, during normal aging, damage to the crystallins leads to their misfolding and the formation of insoluble, "milky white" cataracts. Cataracts are the leading cause of blindness in the world, effecting ~50% of people over age 70. We envisioned that new treatments for cataracts might emerge from a better understanding of how the crystallin proteins interact and how alphaB-crystallin acts as a protective chaperone.

To better understand protein homeostasis in the eye lens, we developed a high throughput screening (HTS) paradigm to identify ligands for alphaB-crystallin. The crystallins are examples of "undruggable" targets, because they lack enzymatic activity, natural ligands or obvious ligand-binding sites. However, using a differential scanning fluorimetry (DSF) platform that was especially adapted for 384-well format, we identified compounds that bind tightly to alphaB-crystallin. We found that these compounds stabilized the alphaB-crystallin dimer, limiting release of free monomer. This mechanism protected alphaB-crystallin from forming cataracts in vitro. Further, these compounds improved the transparency of the lens in mouse models of cataract and in donated human lens tissue, when delivered topically. These results suggest that the dimeric form of alphaB-crystallin is the active chaperone and that it can disaggregate mature cataracts if this activity is enhanced. Using this chemical biology strategy, we are gaining new insights into how protein homeostasis is maintained in the lens and finding new opportunities for treating this common disease.



Nathanael Gray

Nathanael Gray is a Professor of Biological Chemistry and Molecular Pharmacology at Harvard Medical School and Cancer Biology at Dana Farber. His research utilizes the tools of synthetic chemistry, protein biochemistry, and cancer biology to discover and validate new strategies for the inhibition of anti-cancer targets. Dr. Gray's research has had broad impact in the areas of kinase inhibitor design and in circumventing drug resistance. Dr. Gray received his PhD in organic chemistry from the University of California at Berkeley in 1999 after receiving his BS degree with the highest honor award from the same institution in 1995. During his PhD work, Dr. Gray developed new combinatorial chemistry and functional genomics approaches that resulted in the discovery of Purvalanol, one of the first selective inhibitors of cyclin-dependent kinases. After completing his PhD, Dr. Gray was recruited to the newly established Genomics Institute of the Novartis Research Foundation (GNF) in San Diego, California. During his six year stay at GNF, Dr. Gray was rapidly promoted from staff scientist to eventually become the director of biological chemistry where he supervised a group of over fifty researchers integrating chemical, biological and pharmacological approaches towards the development of new experimental drugs. Some of the notable accomplishments of Dr. Gray's team at GNF include: discovery of the first allosteric inhibitors of wild-type and mutant forms of BCR-ABL; discovery of the first selective inhibitors of the Anaplastic Lymphoma Kinase (ALK), an achievement that led to the development of drugs such as LDK378 that have received 'break-through' designation by the FDA for the treatment of EML4-ALK expressing non-small cell lung cancer (NSCLC); and discovery that sphingosine-1-phosphate receptor-1 (S1P1) is the pharmacologically relevant target of the immunosuppressant drug Fingomilod (FTY720) followed by the development of Siponimod (BAF312), which is currently in phase III clinical trials for the treatment of multiple sclerosis. Dr. Gray's generalized strategy for structure-based design of inhibitors that stabilize the inactive kinase conformations (type II) has been widely adopted by the research community and has had a significant impact on the development of numerous inhibitors of tyrosine kinases that are currently undergoing clinical development.

In 2006, Dr. Gray returned to academia and accepted a faculty appointment at the Dana Farber Cancer Institute and Harvard Medical School in Boston. There, he has established a discovery chemistry group that focuses on developing first-in-class inhibitors for newly emerging biological targets, including resistant alleles of existing targets, as well as inhibitors of well-validated targets, such as Her3 and RAS, that have previously been considered recalcitrant to small molecule drug development. His group then uses these novel small molecule inhibitors to investigate the therapeutic potential of these targets in cancer. Due to the productivity of his research program, Dr. Gray was promoted through the academic ranks in record time, achieving full professor status in 2011, five years from when he was hired. His success in developing and translating experimental anti-cancer agents has been due to his highly collaborative and integrated approach to translational research in academia, developing and leading project teams that combine the efforts of world-leading laboratories in structural biology, biochemistry, cancer cell biology, and translational research and then partnering with commercial entities to enable further clinical development. For example, Dr. Gray's development in 2009 of covalent inhibitors of the T790M mutant of EGFR inspired the development of CO-1686, a drug currently in clinical trials in patients with relapsed lung cancer due to resistance to first generation EGFR inhibitors, with results that will be disclosed in the fall of 2013. Dr. Gray has also developed structure-based, generalized approaches for designing drugs to overcome one of the most common mechanisms of resistance observed against most kinase inhibitor drugs, mutation of the so-called "gatekeeper" residue, which has been observed in resistance to drugs targeting BCR-ABL, c-KIT, and PDGFR. This approach to circumventing the T315I gatekeeper mutant of BCR-ABL has been utilized by a new drug, Ponatinib, for the treatment of Chronic Myelogenous Leukemia (CML) that has become resistant to first generation inhibitors. Amongst the additional notable achievements of Dr. Gray's research laboratory are: development of the first ATP-competitive mTOR inhibitor, Torin1, and its use to discover that rapamycin is an incomplete inhibitor of mTOR; development of the first inhibitors of ERK5 (BMK1), which are currently under preclinical development; development of efficient approaches for the discovery of covalent kinase inhibitors that have contributed to the resurgence of interest in this approach; development of the first selective inhibitors of LRRK2, a kinase that is activated by point mutations in a subset of sporadic and familial cases of Parkinson Disease; and discovery of the first potent and selective inhibitors of BCR-ABL, FGFR, MPS1, JNK and BMX which have resulted in new insights into their function and, in several cases, inspired drug discovery efforts. The impact of these new pharmacological agents is amplified by the laboratories approach to 'open-source' discovery: all the compounds are made immediately available to any interested researchers with no strings attached. These contributions have been recognized through numerous awards including the National Science Foundation's Career award in 2007, the Damon Runyon Foundation Innovator award in 2008, the American Association for Cancer Research for Team Science in 2010 and for Outstanding Achievement in 2011 and the American Chemical Society award for Biological Chemistry in 2011.

Developing New Inhibitors for Old Targets: HER3 and KRAS

Nathanael Gray

Part 1: Her3 (ErbB3) is a member of the epidermal growth factor receptor tyrosine kinases that has been well credentialed as a potential anti-cancer target and is thought to represent an “undruggable” small molecule target. Although several therapeutic antibodies directed against Her3’s extracellular domain are currently undergoing clinical testing, to-date there have been no reported small molecule inhibitors directed against the kinase domain of Her3 owing to the proteins classification as a ‘pseudokinase’. An important question for Her3 and the approximately 60 other pseudokinases is whether ATP-competitive small molecules are capable of blocking their biological functions. Here we discuss the development of the first selective Her3 binder, TX1-85-1, that forms a covalent bond with Cys721 located in the ATP-binding site of Her3. We further demonstrate that covalent modification of Her3 exerts minimal effects on Her3-dependent signaling and proliferation of cancer cell lines. Subsequent derivatization of these covalent Her3 ligands with a hydrophobic adamantane moiety demonstrates that the resultant bivalent ligand (TX2-121-1) can inhibit Her3 dependent signaling. Treatment of cells with TX2-121-1 results in partial degradation of Her3 and interferes with productive heterodimerization between Her3 with Her2. Further optimization of the compounds may provide the first pharmacological means of antagonizing Her3-dependent functions in preclinical and clinical models.

Part 2: Cancer-causing mutations of the Ras family proto-oncogenes are common, occurring in 20-30% of all human cancers. Ras operates as a molecular switch, becoming activated when bound to GTP but inactive when bound to GDP. Ras proteins also possess an enzymatic activity for hydrolysis of GTP to GDP. Mutations which diminish this activity result in constitutively activated signaling pathways, leading to uncontrolled cell growth and invasive cancers. Of the Ras family members (H, K, N) K-Ras is most often mutated in cancer and codons 12, 13 and 61 are the most frequently affected. In particular, approximately 30 percent of all incurable lung adenocarcinomas have a K-Ras mutation and, despite the impressive advances in targeted therapies over the past several years, no approved or highly effective targeted therapy exists for this subset of lung cancers. All previous attempts to develop direct-acting Ras drugs have failed to achieve success in the clinic. Oncogenic Ras is arguably the most important cancer target for which no drugs currently exist. Here we describe an approach to overcoming these challenges by developing the first covalent, GTP-site directed inhibitors of K-Ras and demonstrating their efficacy in preclinical models of lung cancer. We will focus on the development of covalent inhibitors of G12C K-RAS, a naturally occurring activating mutation of K-Ras that is present in roughly 10-20% of all Ras-driven cancers with most cases involving lung (most common KRAS mutation in NSCLC), large bowel, biliary tract or endometrium. This mutation places a solvent-accessible cysteine adjacent to the active site, near the usual position of the gamma-phosphate of the native GTP. We have successfully developed a guanosine-analog, SML-8-73-1, that can selectively form a covalent bond to Cys12 and that locks RAS into an inactive conformation. A pro-drug version, SML-10-70-01, where one of the phosphate groups is masked as a cleavable ester, has the ability to inhibit G12C K-RAS-dependent cell growth albeit at high concentrations.



Chuan He

Chuan He is a Professor in the Department of Chemistry and Director of the Institute for Biophysical Dynamics at the University of Chicago. He is also a joint Professor in the Department of Chemical Biology and Director of the Synthetic and Functional Biomolecules Center at Peking University. He was born in P. R. China in 1972 and received his B.S. (1994) from the University of Science and Technology of China. He received his Ph.D. degree from Massachusetts Institute of Technology in chemistry in 2000. After being trained as a Damon-Runyon postdoctoral fellow at Harvard University from 2000-2002, he joined the University of Chicago as an assistant professor, and was promoted to associate professor in 2008 and full professor in 2010. He is also a member of the Cancer Research Center at the University of Chicago. His research spans a broad range of chemical biology, cell biology, molecular biology, biochemistry, structural biology, and genomics. His recent research concerns reversible RNA and DNA methylation in biological regulation. He has been recently selected as an Investigator of the Howard Hughes Medical Institute.

Reversible RNA Methylation in Biological Regulation

Chuan He

Department of Chemistry, The University of Chicago, Chicago, IL 60637

Reversible chemical modifications on nucleic acids and proteins play critical roles in gene expression regulation. The five bases that comprise nucleic acids — adenine, guanine, cytosine, thymine, and uracil — can be chemically and enzymatically modified. These chemical events can have significant biological consequences, particularly for gene expression. Prior to our work, no example of reversible chemical modifications on RNA that could affect gene expression had been shown. Together with our colleagues we have discovered the first two RNA demethylases: FTO and ALKBH5. These two proteins catalyze oxidative demethylation of the most prevalent internal modifications of mammalian messenger RNA (mRNA) and other nuclear RNA, N⁶-methyladenosine (m⁶A). These results indicate that reversible RNA modification could impact biological regulation analogous to the well-known reversible DNA and histone chemical modifications. We have also identified and characterized proteins that can selectively recognize m⁶A-modified mRNA; these m⁶A reader proteins affect the translation status and lifetime of the target mRNA. We have also characterized molecular machines that deposit the m⁶A methylation on nuclear RNA. Our discoveries indicate the presence of a new mode of biological regulation that depends on reversible RNA modification.



Luke D. Lavis

Luke D. Lavis was born in rural southern Oregon. He received his B.S. in Chemistry at Oregon State University in 2000, where he performed undergraduate research in synthetic organic chemistry with James D. White. Dr. Lavis then spent three years in industrial R&D at Molecular Probes in Eugene, OR and Molecular Devices in Sunnyvale, CA. Luke then entered graduate school at the University of Wisconsin–Madison and worked with Ronald T. Raines to develop strategies to trace the path of anticancer proteins in living cells. He received his Ph.D. in Organic Chemistry in 2008. Luke started his independent career as a Fellow at the Howard Hughes Medical Institute’s Janelia Farm Research Campus, and was promoted to Group Leader in 2012. At Janelia, Dr. Lavis works at the interface of chemistry and biology, developing labels for single-molecule imaging, selective enzyme–substrate pairs for targeted molecular delivery, and probes to map cellular activity in intact brain tissue.

Old dyes, new tricks: Building better fluorophores for biology

Luke D. Lavis
Group Leader
Janelia Farm Research Campus, HHMI

Organic fluorophores are invaluable tools for sophisticated biochemical and biological assays. A key advantage of small molecule dyes is the ability to tune and control the properties of the fluorophore using chemistry. Many of the chemical reactions we use to construct dyes are old, however, which limits the collection of accessible fluorophore structures. Our laboratory develops efficient synthetic methodologies to access a broad collection of known and novel fluorophores, including synthetically challenging fluorogenic compounds. In particular, we are interested in combining these facile synthetic strategies with an understanding of fluorophore photophysics to design and build new dyes with improved properties. These probes can be used for a variety of biological experiments, including single-molecule tracking in live cells, super-resolution microscopy, and the discovery of selective enzyme–substrate pairs.



Hening Lin

Dr. Hening Lin was born in China and obtained his B.S. in Chemistry in 1998 from Tsinghua University, Beijing, China. He obtained his PhD degree in 2003 from Columbia University under the guidance of Dr. Virginia Cornish. From 2003-2006, he was a Jane Coffin Childs postdoctoral fellow in Dr. Christopher Walsh's lab at Harvard Medical School. He joined the faculty of Department of Chemistry and Chemical Biology at Cornell University as an assistant professor in 2006. He was promoted to associate professor in 2012 and professor in 2013. His lab works at the interface of chemistry, biology, and medicine. The research in his group focuses on NAD⁺-consuming enzymes that have important biological functions and human disease relevance, including poly(ADP-ribose) polymerases and sirtuins. His lab also works on the biosynthesis of diphthamide, the target of diphtheria toxin. His work is recognized by a Dreyfus New Faculty Award in 2006, the CAPA Distinguished Junior Faculty Award in 2011, and the 2014 ACS Pfizer Award in Enzyme Chemistry.

Regulation of signaling molecules by novel protein posttranslational modifications and sirtuins

Hening Lin

Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853

Sirtuins are a class of enzymes known as nicotinamide adenine dinucleotide (NAD)-dependent deacetylases. Sirtuins regulate aging, transcription, and metabolism, and are important targets for treating several human diseases. There are seven sirtuins in humans, SIRT1-7. Four of them (SIRT4-7) have very weak deacetylase activity, which have caused many confusions and debates in the biological community. My laboratory has recently discovered several novel enzymatic activities, such as desuccinylation and defatty-acylation, for several sirtuins with no robust deacetylase activity. This have led to the identification of previously unknown protein posttranslational modifications and revealed new molecular logics of biology. In particular, we discovered that many signaling molecules are regulated by sirtuin's defatty-acylation activity.



Minkui Luo

Minkui Luo was born in China. He received his B.S. in organic chemistry from Fudan University in 1999. He then traveled to the U.S to pursue his Ph.D in the field of bioorganic and bioinorganic chemistry under the guidance of Dr. John T. Groves, at Princeton. Then, in 2005, I joined the laboratory of Dr. Vern Schramm as a postdoctoral fellow at the Albert Einstein College of Medicine. In 2008, he started his independent career at the Memorial Sloan-Kettering Cancer Center. The current research in the Luo laboratory focuses on developing and implementing chemical tools to define, perturb and manipulate epigenetic functions of protein methyltransferases for disease diagnosis and therapy. Minkui's honors and awards include Outstanding Postdoctoral Research Prize at Albert Einstein College of Medicine, the Basil O'Connor Starter Scholar, NIH Director's New Innovator Award, Alfred W. Bressler Scholar, and the V Scholar Award for Cancer Research. More information on Dr. Luo and his research can be found at <http://www.mskcc.org/research/lab/minkui-luo>.

A Journey of Developing Chemical Tools to Interrogate Protein Methyltransferases

Minkui Luo

Molecular Pharmacology and Chemistry Program, Memorial Sloan-Kettering Cancer Center

Epigenetic regulations are involved in establishing cell-lineage diversity and the errors in these processes have been linked to many diseases including developmental abnormalities, neurological disorders and cancer. Among the key biochemical modifications in epigenetics is protein methylation, a process orchestrated by over 60 human protein methyltransferases (PMTs) with S-adenosyl-L-methionine (SAM) as a cofactor. Defining the targets of the PMTs is pivotal toward elucidating their roles in normal physiology and disease states. Unfortunately, few prior tools were available for mapping proteome-wide and genome-wide methylation events in an unambiguous manner. To address this situation, the Luo laboratory recently developed BPPM (Bioorthogonal Profiling of Protein Methylation) technology for profiling the histone and nonhistone targets of multiple PMTs inside living cells. Here, human SAM synthetase was engineered to process metabolite mimics (terminal-alkyne-containing methionine analogs), thus allowing in situ production of the corresponding SAM analogues. Upon coupling with engineered PMTs, the SAM analogs will be processed to label the histone and nonhistone targets of the corresponding PMTs. The labeled substrates can then be readily enriched via alkyne-azide click chemistry for further analysis. Since only engineered PMTs recognize the SAM analogs, the resultant labeled targets can be assigned unambiguously to the designated (engineered) PMTs. We have successfully implemented the BPPM approach to > 10 human PMTs and showed that each of the PMTs can readily methylate 200 ~ 2000 nonhistone targets, whose functions can associate with most essential biological pathways such as DNA replication, RNA processing, other posttranslational modulars and metabolic enzymes. We will exemplify several findings about unprecedented roles of PMTs such as regulation of transcription factors and RNA splicing factors.

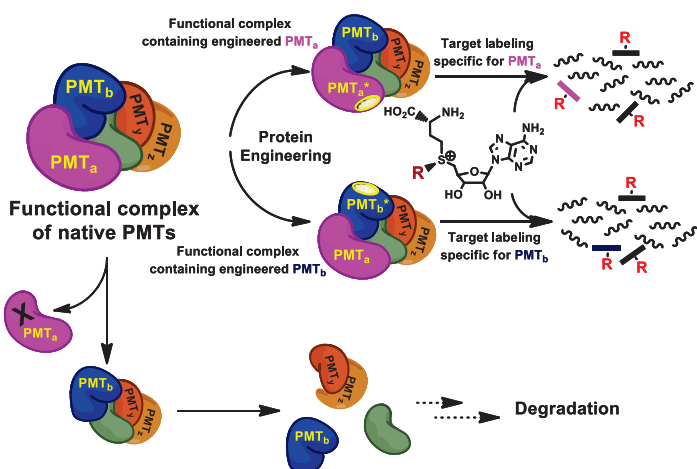


Figure 1. BPPM approach uncover the targets of PMTs.



Dustin J. Maly

Dustin J. Maly was born in Madison, Wisconsin. He received his B.S. in Chemistry from the University of Wisconsin–Madison and his Ph.D. in Chemistry from the University of California, Berkeley. After completing his graduate training, he then moved to the laboratory of Professor of Kevan M. Shokat at the University of California, San Francisco, where from 2003 to 2006 he was a Pfizer Fellow of the Life Sciences Research Foundation (LSRF). Dustin started his independent career in the Department of Chemistry at the University of Washington, Seattle in 2006. He currently is an Associate Professor of Chemistry and Biochemistry, and a member of the Biological Physics, Structure and Design (BMSD) and Molecular and Cellular Biology (MCB) graduate programs. His interests focus on developing new tools for studying signal transduction. Dustin's research program integrates tools from organic synthesis, biochemistry, quantitative proteomics, and cell biology.

Dustin's awards include the Camille Dreyfus Teacher-Scholar Award, the National Science Foundation CAREER Award, and a Sloan Foundation Fellowship. He serves on the editorial board of the *Journal of Biological Chemistry*. More information on Dr. Maly and his research can be found at www.depts.washington.edu/malylab.

Allosteric Modulation of Protein Kinases with Small Molecule Inhibitors

Dustin J. Maly

Departments of Chemistry, 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. For example, the activation loop of the serine/threonine kinase Akt becomes hyper-phosphorylated when its ATP-binding site is occupied by an inhibitor. In addition, a number of ATP-competitive Raf inhibitors lead to activation of the Raf-MEK-Erk signaling pathway, despite inhibition of Raf's kinase 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 the non-catalytic 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 (Figure 1). Furthermore, we have found that conformation-selective inhibition can lead to divergent phenotypic effects in the cell.

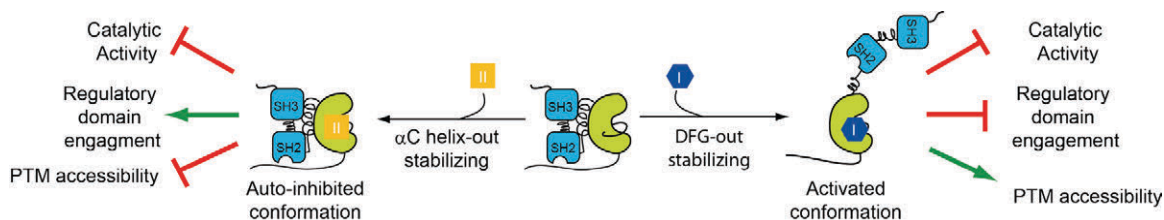


Figure 1. Conformation-selective inhibitors divergently modulate the non-catalytic functions of protein kinases.

An overview of the diverse non-catalytic kinase functions that can be allosterically modulated with ATP-competitive inhibitors will be presented. Furthermore, the molecular logic governing these allosteric effects will be described.



Nicola L. B. Pohl

Nicola L. B. Pohl was born in Montreal, Canada and raised in Georgia and South Carolina. She received her B.A. from Harvard College and her Ph.D. in Chemistry from the University of Wisconsin-Madison. After postdoctoral training in Chemical Biology/Chemical Engineering at Stanford University, she returned to the Midwest to begin her independent career at Iowa State University in 2000. In 2012, she moved to Indiana University-Bloomington, where she currently holds the Joan and Marvin Carmack Chair in Bioorganic Chemistry. She also serves as the Co-director for the Chemical Biological Interface Training Program. Her interdisciplinary research interests focus on understanding the unique properties of carbohydrates in order to develop chemical, enzymatic, and analytical tools to study their biological functions.

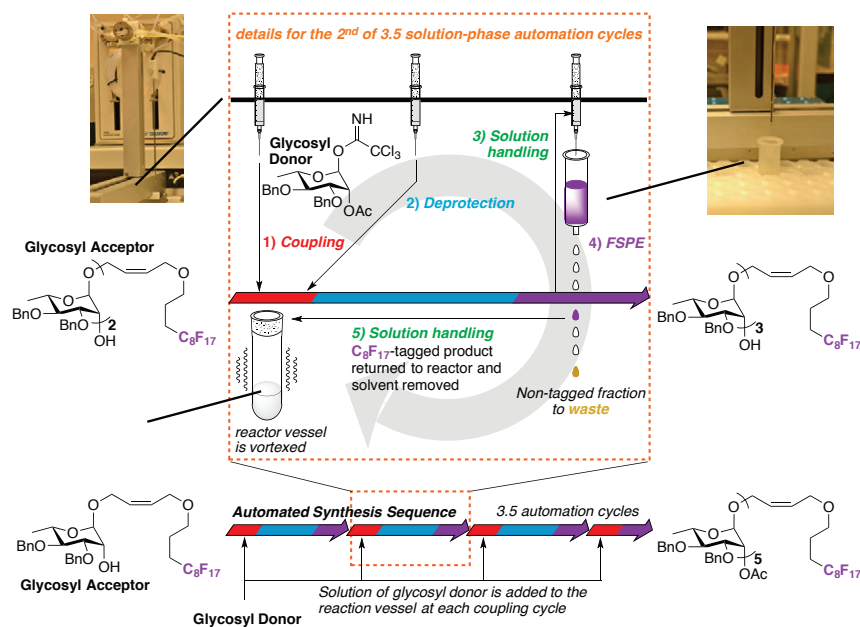
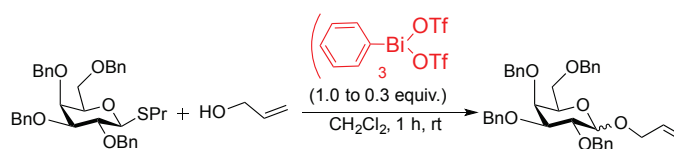
Pohl's honors and awards include the National Science Foundation National CAREER Award, a Sloan Foundation Fellowship, a Cottrell Scholar Award from the Research Corporation for Science Advancement, and the Horace S. Isbell Award from the Carbohydrate Division of the American Chemical Society. She has served on several editorial boards, currently including *The Journal of Organic Chemistry*. She is currently functioning as Past Chair of the Carbohydrate Division of the American Chemical Society. More information on Dr. Pohl and her research can be found at <http://www.indiana.edu/~pohllab/index.html>.

The Challenges of Automated Oligosaccharide Synthesis for the Development of Tools for Chemical Biology

Nicola L. B. Pohl

Department of Chemistry, Indiana University-Bloomington

Many advances in understanding the role of carbohydrates in biological systems are stalled by the lack of diverse and chemically well-defined glycan structures. For automation to play as vital a role in the synthesis of oligosaccharides as it currently does in peptide and nucleic acid production, the major bottleneck of building block access must be surmounted. One way to shorten the synthesis of the required monomers is by the use of thioglycosides, since the anomeric thiol linkage can be carried through a variety of protection/deprotection reactions to selectively block the remaining hydroxyl functional groups prior to activation of the sulphur linkage. Unfortunately, thioglycoside activation procedures either are not inert to the alkenes contained in our fluororous linker used to automate the iterative oligosaccharide synthesis or require a mixture of activating reagents that make the method less amenable to automated liquid handling protocols. We recently discovered that a pentavalent bismuth compound could circumvent these issues, however, and successfully activate a thioglycoside. Preliminary data show that a solution of this activator can also be used for the automated synthesis of a glycosyl linkage on our automated solution-based oligosaccharide synthesis platform to complement our current strategy outlined below using Schmidt trichloroacetimidate chemistry. An overview of this strategy and its applications to vaccine and vaccine adjuvant design will be presented.





Frank C. Schroeder

Frank C. Schroeder grew up in Hamburg, in Northern Germany. He studied chemistry and physics at the University of Hamburg, where he worked with Prof. Wittko Francke. He received his Doctorate in 1998 for studies of structures and functions of insect-derived natural products. In 2004, he joined Jon Clardy's group at Harvard Medical School as the Director of the Natural Products Initiative, before starting his independent career as an Assistant Professor at Cornell's Boyce Thompson Institute (BTI) in August 2007. Since 2013, he is Associate Professor at BTI and Adjunct Professor in the Department of Chemistry and Chemical Biology.

Schroeder's research is directed at characterizing structures and functions of biogenic small molecules, combining expertise in molecular biology, analytical chemistry, and organic synthesis. Based on combining innovative NMR-spectroscopic methodology with genetic approaches, his lab initiated a comprehensive effort to characterize structures and functions of the metabolome of the model organism *Caenorhabditis elegans*, focusing on conserved signaling cascades that control development, aging, and social behaviors. Complementing his work on nematode model organisms, his lab has developed NMR-based comparative metabolomics as a new strategy for identifying the small-molecule products of cryptic PKS and NRPS gene clusters in bacteria and fungi, focusing in particular on the identification of virulence factors

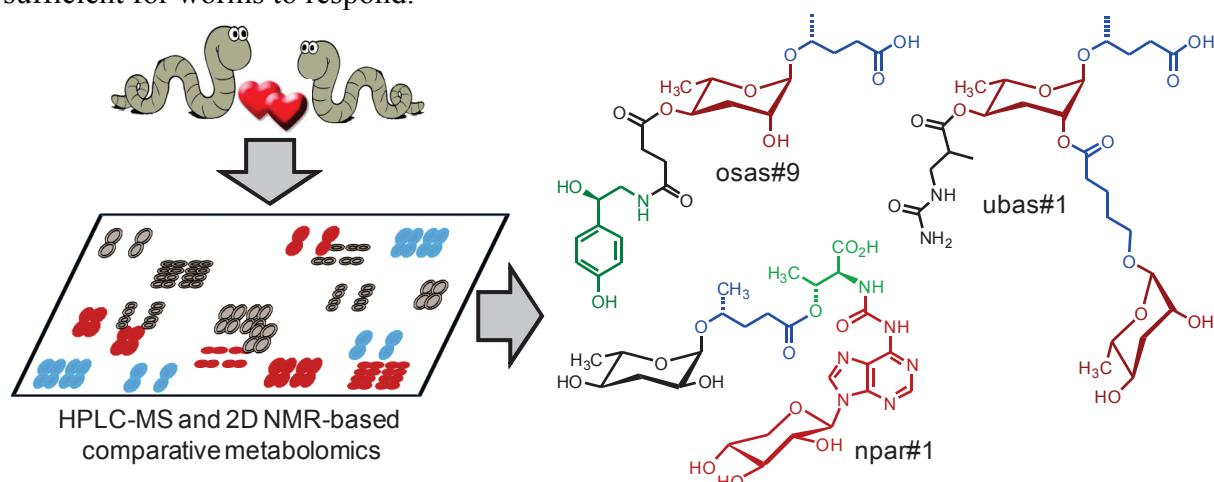
Worm language: a natural combinatorial library and a xylopyranose-based nucleoside

Frank C. Schroeder

Boyce Thompson Institute and Depart. of Chemistry and Chemical Biology, Cornell University

The nematode *Caenorhabditis elegans* is one of the most important model organisms for biomedical research, because of its biological tractability and because many of its physiological pathways show strong homology to corresponding pathways in humans. We aim to complement the highly developed genomics and proteomics of *C. elegans* with a comprehensive structural and functional characterization of its metabolome, which has been explored to only a very limited extent.

We found that worms are amazingly skilled chemists: using simple building blocks from conserved primary metabolism and a strategy of modular assembly, *C. elegans* and other nematode species create complex molecular architectures to regulate almost every aspect of their development and behavior. Phenotypes regulated by this combinatorial library of small-molecule signals include dauer formation, adult phenotypic plasticity, adult lifespan, attraction of the other sex, aggregation, dispersal, and other behaviors. Most of the identified compounds are based on the dideoxysugars ascarylose or paratose, which serve as scaffolds for attachment of moieties from amino acid, carbohydrate, neurotransmitter, lipid, and nucleoside metabolism, including an unusual xylopyranose-based adenosine derivative. The resulting signaling molecules can be active at femtomolar concentrations, such that encountering just a few molecules per minute is sufficient for worms to respond.



Motivated by this unexpected structural and functional diversity, we have embarked on a systematic characterization of the *C. elegans* metabolome combining mutant screens and 2D NMR/HPLC-MS-based comparative metabolomics. We find that most small molecules in *C. elegans* have remained undescribed and that even important primary metabolite classes may include non-canonical compounds. Their identification and quantification in genome-wide mutant screens will, akin to transcriptional profiling, provide a new basis for the study of metabolism and evolutionarily conserved signaling pathways in this model organism. Moreover, the identification of many new variants of primary metabolism-derived structures that serve important signaling functions in *C. elegans* provides a strong incentive for a comprehensive re-analysis of metabolism in higher animals, including humans.



Sachdev S. Sidhu

Sachdev Sidhu studied chemistry at Simon Fraser University and obtained his B.Sc. with honors in 1991. He then continued his graduate work at Simon Fraser University where he investigated enzyme function, and obtained his Ph.D. in 1996. Following a postdoctoral research fellowship with James Wells at Genentech, Dr. Sidhu joined the Protein Engineering department as a principal investigator in 1998. In 2008, Dr. Sidhu moved to the University of Toronto, where he is a professor in the Banting and Best Department of Medical Research and the Department of Molecular Genetics. He has published more than 100 scientific papers and is a co-inventor on more than 30 patents filed with the US patent office. Dr. Sidhu's research interests focus on the use of combinatorial biology methods to explore protein structure and function, and his group is currently developing synthetic antibody libraries and other scaffolds as sources of potential therapeutics.

Modulation of Cell Signalling with Synthetic Proteins

Sachdev S. Sidhu

Department of Molecular Genetics, University of Toronto

Affinity reagents that target and modulate proteins are of crucial importance for both basic research and therapeutic development. To date, antibodies derived by animal immunization have been the dominant source of affinity reagents, but in recent years, research in protein engineering has given rise to a new wave of technologies that promise to transform the field. “Synthetic antibodies” use man-made antigen-binding sites and thus circumvent the need for immune repertoires. We have developed simple synthetic antibodies that use a single human framework and limited chemical diversity in restricted regions of the antigen-binding site. Moreover, the use of synthetically designed libraries enables the use of alternative scaffolds for applications beyond the reach of the antibody framework. In particular, we have designed libraries of ubiquitin variants that can be used to inhibit or activate virtually any of the hundreds of ligase and deubiquitinating enzymes in the ubiquitin system. These ubiquitin variants are adapted for intracellular function, and thus, they can be introduced into cells to probe function in a living cellular context. In addition, we have developed small, optimized scaffolds that function like antibodies but are amenable to full chemical synthesis, thus enabling the incorporation of non-natural amino acids. The power of the technology has been demonstrated by the development of potent protein inhibitors composed entirely of D-amino acids. In sum, these advances in the design of synthetic binding proteins extend the applications for affinity reagents well beyond the range of natural antibodies and this should have a transformative effect on many areas of biological research.



David A. Spiegel

David A. Spiegel was born in New York City, and grew up in Teaneck, New Jersey. From a very young age, he was fascinated by the chemistry and biology of small molecules, and at 16 began doing research in a neuroanesthesiology laboratory at the University of Iowa. He went on to attend Harvard University where he conducted research under the guidance of Professor Yoshito Kishi. After graduating from Harvard, David began in Yale University's M.D./Ph.D. program. There he conducted graduate research in Professor John Wood's laboratory focusing on developing synthetic approaches toward the phomoidrides. During the course of these studies, he discovered that trialkylborane-water complexes could function as H-atom donors in free radical reactions. Following graduation from Yale, Professor Spiegel moved back to Harvard for postdoctoral studies under the guidance of Professor Stuart L. Schreiber. There he focused on developing an oligomer-based method for small-molecule synthesis to enable the rapid assembly of skeletally diverse small molecules starting from simple monomers.

David began as an Assistant Professor at Yale in June of 2007. Since that time he has been fortunate to be named recipient of the NIH New Innovator Award, the Ellison Medical Foundation New Scholar Award, the Camille and Henry Dreyfus New Faculty Award, and an Alfred P. Sloan Fellowship.

Using Small Molecules to Engineer and Explore Human Immunity

David A. Spiegel, Ph.D., M.D.,

Associate Professor of Chemistry and Pharmacology, Yale University

Antibody-based therapeutics have become critical instruments in treating diseases ranging from rheumatoid arthritis to cancer in recent years. However, antibodies and other therapeutic proteins are limited in therapeutic applications by their chemical structures: because they are peptide-based, they require intravenous administration, are often highly immunogenic or allergenic, and treatment regimens are often very costly.

This talk describe recent research efforts in our laboratories toward the design, chemical synthesis, and biological characterization of small molecule antibody recruiting therapeutics against prostate cancer, *Staphylococcus aureus*, and the human immunodeficiency virus (HIV). These are bifunctional small molecules designed to redirect antibodies already present in the human bloodstream to the surfaces of pathogenic cells, such as cancer cells, bacteria, and virus particles. The ternary complex formed between these agents, endogenous antibodies, and target cells will lead to immune-mediated pathogen destruction. In theory, this strategy would exploit many of the advantages of biologics, while circumventing the disadvantages, by capitalizing on the chemical properties of small molecules (e.g., high oral bioavailability, facile synthesis, and low cost).

It is our hope that this small molecule-based strategy will serve as starting point toward entirely novel scientific insights and therapeutic approaches relevant to a wide range of disease states.



Alexander V. Statsyuk

Alexander V. Statsyuk graduated from the Moscow State University, Russia in 1998, with the BS degree in chemistry. In January of 2001 Alexander moved to the USA to pursue his PhD studies at the University of Chicago under the direction of Prof. Sergey A. Kozmin, where he worked on the total synthesis and target identification of the cytotoxic natural product bistramide A.

Subsequent to his Ph.D. work Alexander moved to UCSF to conduct his postdoctoral studies with Prof. Kevan Shokat (UCSF). Since 2010, Alexander V. Statsyuk has been an Assistant Professor in the Department of Chemistry at Northwestern University, where he directs his independent research program aimed at studying the ubiquitin-proteasome system.

The Ubiquitin System: Challenges, Opportunities, and Serendipitous Discoveries

Alexander V. Statsyuk, Chemistry Department, Northwestern University

The ubiquitin system controls all aspects of eukaryotic biology, and provides unique opportunities to advance human medicine. Protein ubiquitination is controlled by the cascade of E1, E2 and E3 enzymes that eventually leads to poly- or mono-ubiquitination of protein substrates leading to different physiological outcomes (Figure 1).

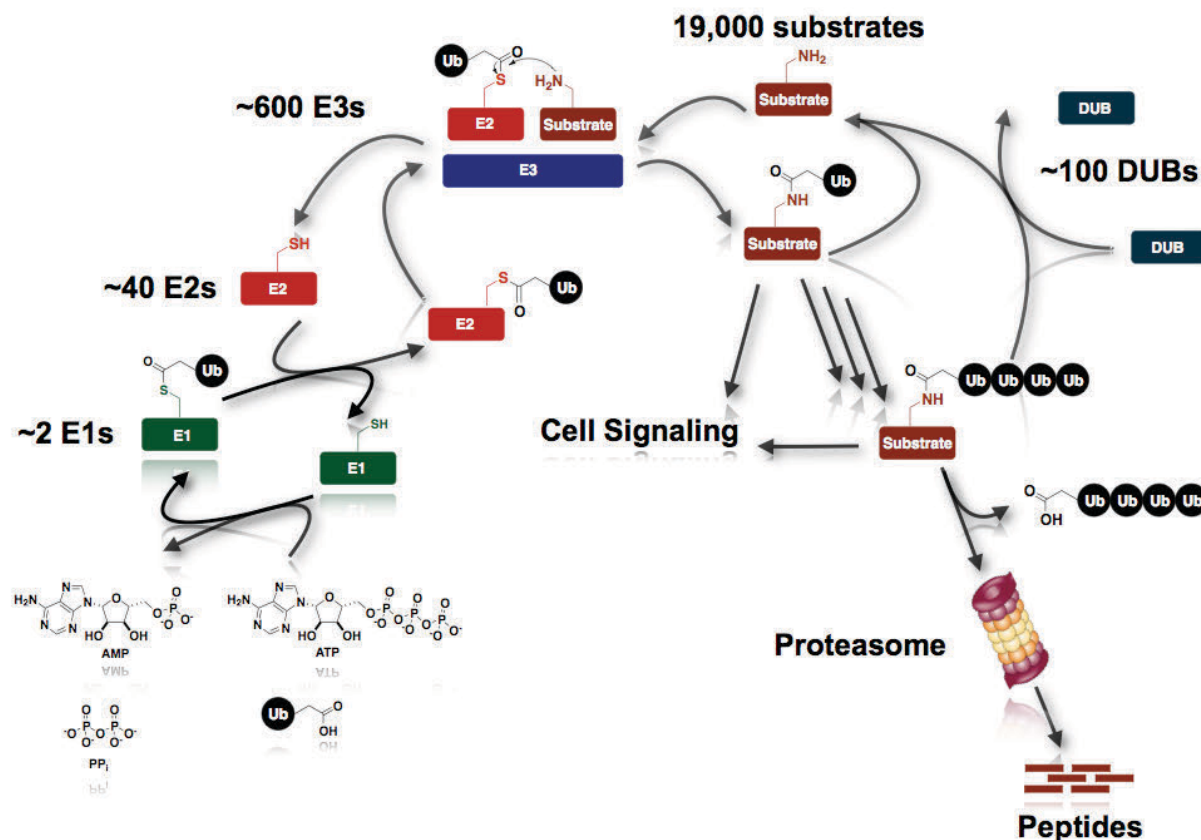


Figure 1. An overview of the ubiquitin system.

To study the ubiquitin system we first developed technologies that include: 1) activity based probes for E1 enzymes, 2) small molecule inhibitors of E1 enzymes, 3) cysteine scanning method to study E2/E3 interaction *in vitro*, 4) irreversible tethering technology (considered impossible) to discover small molecule inhibitors of E1, E2, E3, and de-ubiquitinating enzymes, and 5) protein ubiquitination without ATP, E1, and E2 enzymes, which provides simple assays to screen for E3 inhibitors. With these tools in hands we transitioned to the second phase of our research program aimed at providing new therapeutic leads to treat human diseases.

We will discuss the use of irreversible tethering technology to discover covalent small molecule inhibitors of E3 enzyme Nedd4-1 (considered undruggable), which is oncogenic and is known to ubiquitinate and degrade tumor suppressors PTEN. As we expected, when we applied impossible technology (irreversible tethering) to undruggable protein target (Nedd4-1) we have made a serendipitous discovery: an inhibitor of Nedd4-1 enzyme processivity and not its catalytic activity. This discovery illuminated important aspects of PTEN biology, which we did not know about. Excitingly, we were able to obtain the crystal structure of Nedd4-1 bound to small molecule inhibitor for further optimization studies. Finally we will outline future directions.



Yi Tang

Yi Tang received his undergraduate degree in Chemical Engineering and Material Science from Penn State University. He received his Ph.D. in Chemical Engineering from California Institute of Technology under the guidance of Prof. David A. Tirrell. After NIH postdoctoral training in Chemical Biology from Prof. Chaitan Khosla at Stanford University, he started his independent career at University of California Los Angeles in 2004. He is currently the Chancellor Professor at the Department of Chemical and Biomolecular Engineering at UCLA, and holds joint appointments in the Department of Chemistry and Biochemistry; and Department of Bioengineering. His lab is interested in identifying new enzymes from the biosynthetic pathways of polyketides, nonribosomal peptides, terpenoids, alkaloids and hybrid compounds. His group has mined numerous cryptic natural products from fungal species, including potential immunosuppressant polyketides from pathogenic fungi. His group is also interested in combining enzyme discovery and protein engineering towards the green synthesis of important pharmaceuticals. They demonstrated the potential of this approach by establishing a biocatalytic approach for making the blockbuster drug simvastatin.

His recent awards include the American Institute of Chemical Engineers (AIChE) Allan P. Colburn Award (2009), the Young Investigator Award from the Society of Industrial Microbiology (2010), the American Chemical Society (ACS) Biochemical Technology Division (BIOT) Young Investigator Award (2011), the ACS Arthur C. Cope Scholar Award (2012), the EPA Presidential Green Chemistry Challenge Award (2012), NIH DP1 Director Pioneer Award (2012) and the ACS Eli Lilly Award in Biological Chemistry (2014).

Exciting New Enzymes from Fungal Biosynthetic Pathways

Yi Tang

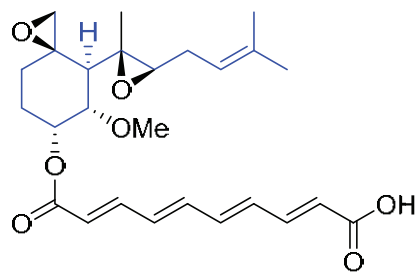
Department of Chemical and Biomolecular Engineering

Department of Chemistry and Biochemistry

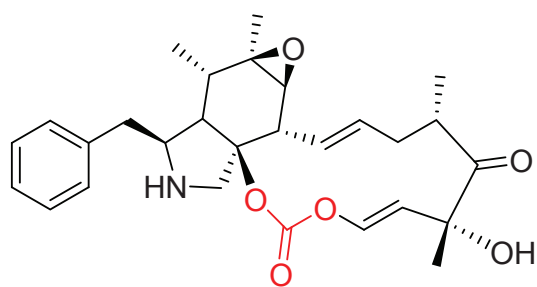
Department of Bioengineering

University of California, Los Angeles

Natural products biosynthesized by filamentous fungi exhibit complex structures and exciting biological activities. For example, fumagillin and cytochalasin E produced from *Aspergillus* species are potent inhibitors of methionine aminopeptidase and angiogenesis, respectively. Both compounds have been considered for anticancer therapies. The biosynthesis mechanisms of fumagillin and cytochalasin E have remained relatively unexplored from genetic and biochemical perspectives. In this presentation, we will focus on the elucidation of the biosynthesis of these two compounds to illustrate the potential for finding new enzymes from fungal biosynthetic pathways. First, we will talk about the identification of a new membrane bound type I terpene cyclase that converts farnesyl pyrophosphate into *trans*- β -bergamotene in the fumagillin pathway. This is the first example of an integral membrane enzyme catalyzing terpene cyclization through ionization of the allylic diphosphate ester to generate an allylic cation. Then we will talk about a fascinating flavin-dependent monooxygenase that oxidizes a ketone intermediate in the cytochalasin E pathway into a carbonate-containing product. This is the first example of enzymatic carbonate formation in nature. Several possible mechanisms for the ketone to carbonate transformation will be discussed.



Fumagillin



Cytochalasin E



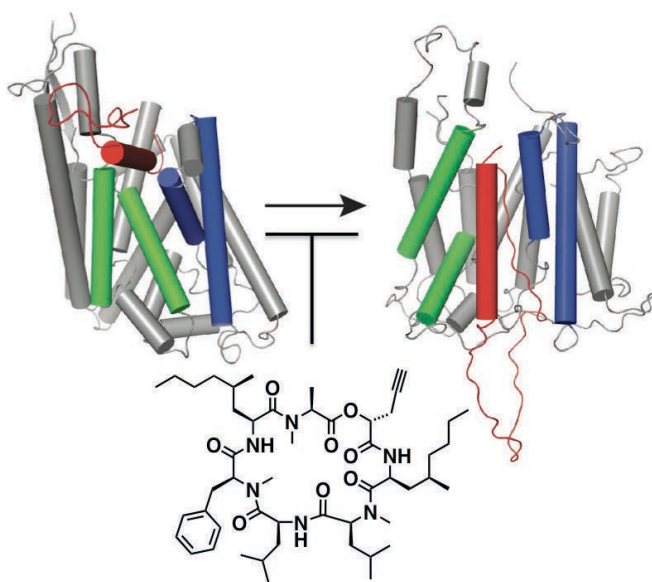
Jack Taunton

Jack Taunton was born in Madison, WI. He obtained his undergraduate degree from Trinity University in San Antonio, TX and received a Ph.D. in chemistry from Harvard University. As a graduate student with Stuart Schreiber, Jack identified and cloned the first histone deacetylase (HDAC1) as the molecular target of trapoxin, a cyclic peptide natural product. He moved to Tim Mitchison's lab at Harvard Medical School for postdoctoral studies, where he studied the biochemistry underlying actin-dependent cell motility. His current research focuses on the design and discovery of chemical tools to modulate protein function, with the aim of demystifying complex cellular processes relevant to human disease. Areas of interest include the structure-based design of reversible and irreversible covalent probes, as well as mechanistic studies of biologically active natural products. His lab also studies the mechanisms by which multiprotein complexes assemble on membrane surfaces to control cytoskeletal and membrane dynamics. He is a professor in the department of cellular and molecular pharmacology at the University of California, San Francisco, and a Howard Hughes Medical Institute Investigator.

Selective small-molecule modulators of secretory and membrane protein biogenesis

Jack Taunton

Most secreted and integral membrane proteins in human cells are co-translationally targeted to the endoplasmic reticulum (ER). This process begins when a hydrophobic signal sequence emerges from a translating ribosome. After binding the signal recognition particle, the ribosome-nascent polypeptide complex is targeted to the Sec61 translocon, a multisubunit protein machine embedded in the ER membrane. Sec61 acts as a protein-conducting channel that opens toward the ER lumen to mediate co-translational translocation of nascent secretory proteins. In addition, a gate in Sec61 opens laterally to mediate transmembrane domain insertion into the lipid bilayer. The mechanism of Sec61 gating and the role of the hydrophobic signal sequence in this highly dynamic process are poorly understood.



Inspired by the fungal natural product HUN-7293, we discovered the cotransin cyclic depsipeptides, which bind directly to Sec61 and inhibit the co-translational translocation of a subset of secretory and membrane proteins. Remarkably, the sensitivity of a given secretory protein to cotransin is determined by its signal sequence. How cotransin discriminates among thousands of Sec61-dependent clients by virtue of their divergent signal sequences is completely mysterious. In this talk, I will describe our recent studies aimed at unraveling cotransin's mechanism of action. In addition to providing new insights into how the Sec61 translocon works, our studies suggest potential therapeutic applications for substrate-selective Sec61 modulators.



Paul R. Thompson

Paul R Thompson was born in Toronto, ON, Canada. He received both his B.S.c. and Ph.D. in Biochemistry from McMaster University in Hamilton, ON, Canada. After postdoctoral training with Philip Cole in the Department of Pharmacology at the Johns Hopkins University School of Medicine, he then moved to the Department of Chemistry and Biochemistry at the University of South Carolina to begin his independent career. In May 2010, he moved to The Scripps Research Institute in Jupiter where he is an Associate Professor of Chemistry. Paul's interdisciplinary research program focuses on the development of novel therapeutics for a range of diseases including cancer, rheumatoid arthritis, inflammatory bowel disease, and lupus. In particular, he has developed a novel series of chemical probes to study the enzymes that catalyze protein post-translational modifications. Among these enzymes, he has a particular interest in the enzymes that methylate, phosphorylate and citrullinate arginines. His research combines tools from organic synthesis, protein chemistry, enzymology, and structural biology.

Thompson's honors and awards include the Camille Dreyfus Teacher Scholar Award, the 2010 South Carolina Governor's Young Scientist Award for Excellence in Scientific Research, he was a New Investigator of the American Heart Association, and he received a postdoctoral fellowship from the Canadian Institutes for Health Research. He also received a National Science and Engineering Research Canada Graduate Fellowship and graduated *Summa Cum Laude* from McMaster University. Paul is a co-founder and member of the scientific advisory board of Padock Therapeutics and is the current chair of the 2014 Enzymes, Coenzymes & Metabolic Pathways Gordon Research Conference. More information on Dr. Thompson and his research can be found at: <http://www.scripps.edu/thompson/index.html>

Picking the PADlock: Chemical Probes targeting Protein Arginine Deiminase activity

Paul Thompson

The Protein Arginine Deiminases (PADs) are a small family of human enzymes that catalyze the post-translational hydrolysis of arginine residues to generate citrulline (Figure 1). These enzymes are important regulators of gene transcription and their activity is upregulated in multiple inflammatory diseases, including rheumatoid arthritis, ulcerative colitis, and cancer.

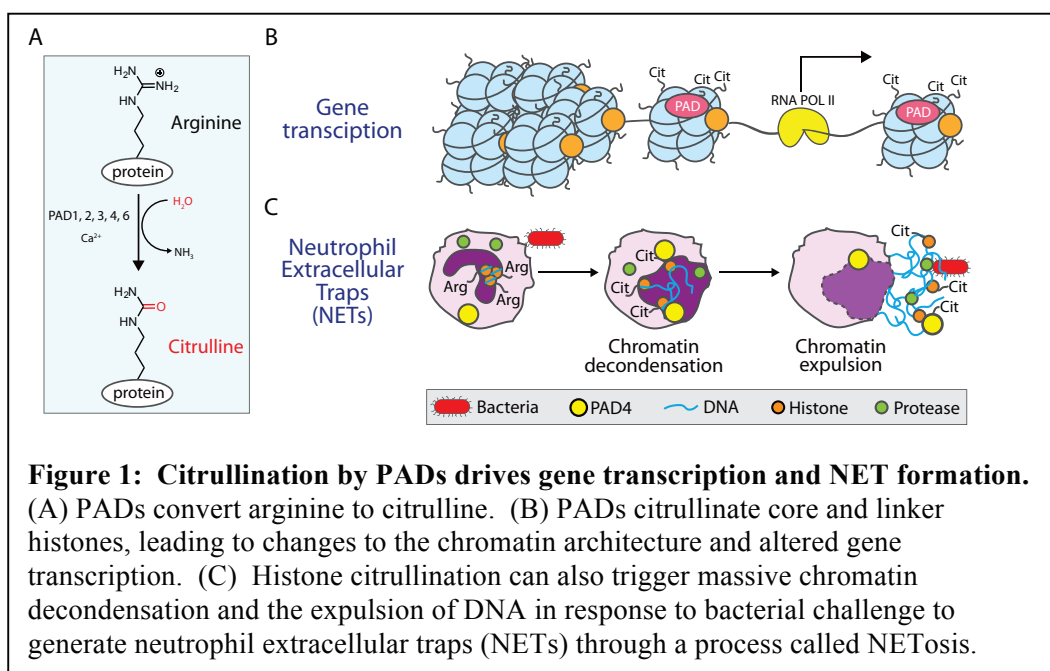


Figure 1: Citrullination by PADs drives gene transcription and NET formation.

(A) PADs convert arginine to citrulline. (B) PADs citrullinate core and linker histones, leading to changes to the chromatin architecture and altered gene transcription. (C) Histone citrullination can also trigger massive chromatin decondensation and the expulsion of DNA in response to bacterial challenge to generate neutrophil extracellular traps (NETs) through a process called NETosis.

Herein, I will discuss our efforts to develop chemical probes targeting the PADs and protein citrullination, focusing on our success in validating the PADs as therapeutic targets, as well as our more recent efforts to develop phenylglyoxal based reagents that can be used to readily visualize protein citrullination and identify disease biomarkers. Additionally, I will describe our efforts, both crystallographic and enzymological, to understand how calcium regulates the activity and nuclear localization of PAD2.



Eranthie Weerapana

Eranthie Weerapana was born in Sri Lanka and received her B.S. in Chemistry from Yale University. She received her Ph.D. in Chemistry from MIT, where she worked with Professor Barbara Imperiali, investigating glycosyltransferases involved in N-linked glycosylation in the gram negative bacterium *Campylobacter jejuni*. She then performed postdoctoral studies at The Scripps Research Institute, La Jolla where she worked with Professor Benjamin F. Cravatt to develop chemical proteomic methods to investigate reactive cysteines in complex proteomes. Eranthie started her independent research career in 2010 at Boston College, where she is currently an Assistant Professor of Chemistry. Her interdisciplinary research program focuses on applying mass spectrometry methods to identify regulatory cysteine residues in the human proteome, and chemical biology approaches to develop covalent small-molecule modulators for these cysteine-mediated protein activities. Her research group combines tools from organic synthesis, cell and molecular biology and mass spectrometry-based proteomics.

Eranthie's awards include a Smith Family Award for Excellence in Biomedical Sciences (2011) and a Damon Runyon-Rachleff Innovation Award (2012). More information on her research can be found at <https://www2.bc.edu/eranthie-weerapana/>.

Chemical-proteomic methods to identify nitrosation-sensitive cysteines

Eranthie Weerapana

Department of Chemistry, Boston College

Cysteine residues on proteins play numerous functional roles as catalytic nucleophiles, redox-active disulfides, or metal-binding residues. Outside of their direct role in catalysis, cysteines can also regulate protein activity via posttranslational modification by endogenous electrophiles and reactive oxygen species (Figure 1). These modified cysteine residues do not conform to a conserved sequence or structural motif, rendering the global identification of regulatory cysteines a considerable challenge. We hypothesize that cysteines that are hypersensitive to different oxidants or nitrosating agents are likely to be enriched in functional residues that serve to modulate protein activity. To explore this hypothesis, we focused our initial efforts on investigating cysteine susceptibility to nitrosation. S-nitrosation is a unique posttranslational modification that serves to temporally and spatially control protein activity and localization. In order to identify cysteine residues hypersensitive to nitrosation, we developed a chemical-proteomic platform that ranks cysteines in the human proteome by sensitivity to a variety of nitric oxide donors. Our proteomic studies identified several known sites of nitrosation, as well as previously unannotated cysteines. We then proceeded to functionally characterize several of the unannotated cysteines using a variety of cell and molecular biology approaches. We show that many of these cysteines are located distal from the active site, and yet mediate essential protein functions such as substrate binding and localization. Overall, our studies aim to unearth novel functional cysteine residues in the proteome, with the long-term goal of identifying new modes of protein regulation through reactive cysteines.

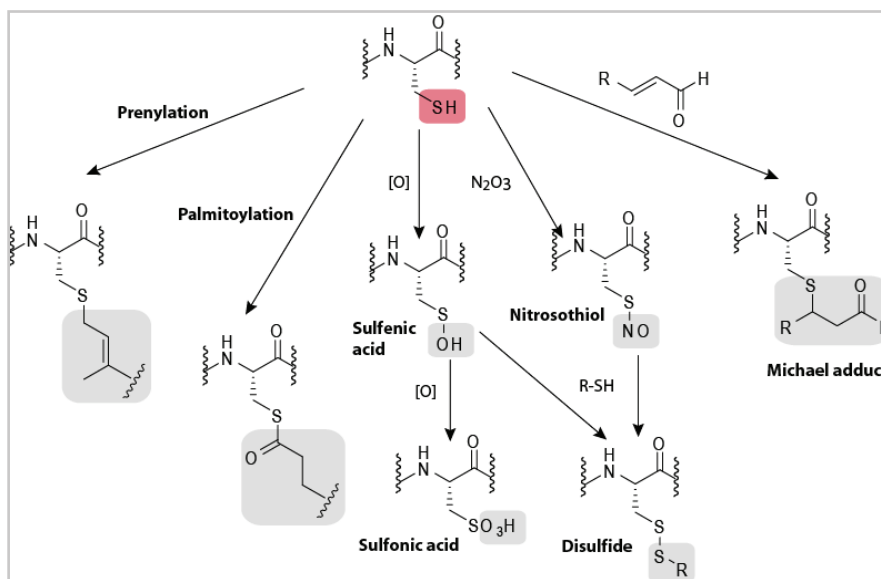


Figure 1: Cysteine residues are susceptible to diverse posttranslational modifications.



Douglas B. Weibel

Douglas B. Weibel was born in Philadelphia, PA. He received his B.S. degree in chemistry in 1996 from the University of Utah (with Prof. C. Dale Poulter). From 1996-1997 he was a Fulbright Fellow at Tohoku University, Japan where he studied organometallic chemistry (with Prof. Yoshinori Yamamoto). He received his M.S. (1999) and Ph.D. (2002) from Cornell University (with Prof. Jerrold Meinwald) for research in the fields of organic and analytical chemistry. During his graduate studies he was an intern at Orchid Biosciences Inc. (now Orchid Cellmark) and a visiting scientist at the Max Planck Institute for Chemical Ecology, Jena, Germany (with Prof. Wilhelm Boland). From 2002-2006 he was an NIH NRSA postdoctoral fellow at Harvard University (with Prof. George M. Whitesides) where his research spanned the fields of chemistry, engineering, and microbiology. He was a student in the 2005 Physiology Course ('Modern Cell Biology using Microscopic, Biochemical, and Computational Approaches') at the Marine Biological Laboratory at Woods Hole (Course Directors, Prof. Ron Vale and Prof. Tim Mitchison). He is currently an Associate Professor of Biochemistry, Chemistry, and Biomedical Engineering, an affiliate of the Genome Center, and a trainer in the Biophysics Program, Biotechnology Training Program, the Cellular and Molecular Biology Program, the Chemistry and Biology Program, the Materials Science Program, the Molecular Biosciences Training Program, and the Microbiology Doctoral Training Program, all at the University of Wisconsin-Madison. On sabbatical for one year starting in June 2014, he will be a visiting professor at Google[x] and Stanford University. His research interests span the fields of biochemistry, biophysics, chemistry, materials science and engineering, and microbiology. More information on his research can be found at: <http://www.WeibelLab.org>.

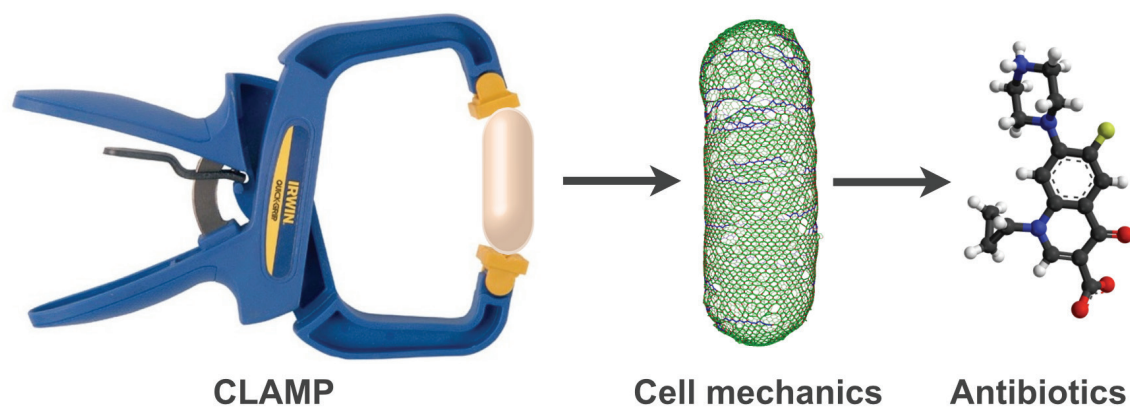
Mechanical genomics: genome-wide identification of regulators of bacterial cell mechanics

Douglas B. Weibel

Departments of Biochemistry, Chemistry, and Biomedical Engineering
University of Wisconsin-Madison

The peptidoglycan (PG) consists of a single, cross-linked layer of polysaccharide that forms the primary load-bearing material of bacteria and resists the large osmotic pressure across the cell wall. As defects in PG assembly and remodeling are catastrophic to cells, mechanistic studies in this area may lead to the discovery of new targets and antibiotics that inhibit cell wall assembly.

In the first part of my talk, I describe a chemistry-based approach we have developed to measure the stiffness of the cell wall of growing bacteria. We refer to this technique as CLAMP: cell length analysis of mechanical properties, as it effectively describes a polymer clamp technique for determining the composite Young's modulus for the cell wall (E_{cell}). Importantly, E_{cell} can be translated into changes in PG mechanical properties and provide insight into several properties of this material, including: 1) its structure; 2) its conservation across Eubacteria; and 3) its response to chemical and biological perturbations. In the second part of my talk I describe the application of a high-throughput variation of CLAMP to study *mechanical genomics*—that is, quantifying how genome-wide alterations influence cell mechanical properties. We have used CLAMP to screen an entire gene deletion library in *Escherichia coli* (~4000 mutants) and have correlated the absence of each gene with cell wall mechanical properties. This approach has enabled us to identify new machinery and regulators of PG and cell wall assembly and has yielded some very surprising results. I describe the results of these experiments and how it is shaping our view of the assembly, properties, and function of the cell wall and providing targets for antimicrobial chemotherapy.



Poster Presenter Index

Presenter	Poster Title	Abstract Page	Board Number
Heeseon An	Development of Activity-Based Probes for Ubiquitin and Ubiquitin-Like Protein Signaling Pathways	64-65	Friday 1
Gene E. Ananiev	Early Drug Discovery Services at UW-Madison Small Molecule Screening and Synthesis Facility	66	Friday 2
Kristen A. Andersen	Differential Labeling of Cell Surface Glycans with Diazo and Azido Sugars	67	Friday 3
Matthew R. Aronoff	Protection and Detection of Boronic Acids for Chemical Biology	68	Friday 4
Nicole A. Beauchene	Influence of O ₂ on the Ferric Uptake Regulator (Fur) Regulon in <i>Escherichia coli</i> K12	69	Friday 5
Nitasha Bennett	Dissecting Immune Signaling with Synthetic Multivalent Ligands	70	Friday 6
Rachel C. Botham	Small-molecule Priming of Procaspase-3 Lowers the Apoptotic Threshold of Cancer Cells	71	Friday 7
Michelle Boursier	Elucidating the Mechanisms by which Non-native Acylated L-Homoserine Lactones Modulate LuxR-type Quorum Sensing Receptors in Bacteria	72	Friday 8
Benjamin M. Brandsen	DNA-Catalyzed Hydrolysis of Esters and Aromatic Amides	73	Friday 9
Robert A. Brown	Chemical Tools to Study and Exploit Bacterial Cell Wall Assembly	74	Friday 10
Phillip Calabretta	Chemical Complementation of Corynebacterial Arabinan in a Deficient Mutant	75	Friday 11
Ross W. Cheloha	Designing Parathyroid Hormone Mimics with Enhanced Properties via Sequence-Based α/β -Peptide Design	76	Friday 12
Nicholas Clay	"Breaking Collagen": Regulating <i>In Vitro</i> HepG2 Malignancy with <i>In Situ</i> MMP Degradation of Collagen-PEG Hydrogels	77	Friday 13
Brett Covington	Metabolomics for Antibiotic Discovery and Mechanistic Characterization	78	Friday 14
Dahvid Davda	Acyl Protein Thioesterase 2 Regulates MAPK/ERK Signaling and Cell Polarity	79	Friday 15
Kathryn Del Vecchio	Functional Studies of Ebola Virus Matrix Protein VP40	80	Friday 16

Presenter	Poster Title	Abstract Page	Board Number
Lisa Della Ripa	Investigating Sterol-Amphotericin B Interactions by Solid-State NMR Spectroscopy	81	Friday 17
Stephanie Dillon	Nickel Superoxide Dismutase: Spectroscopic and Computational Evaluation of the Roles Played by the Secondary and Tertiary Sphere Residues Glu17 and Arg47	82	Friday 18
Emily Dykhuizen	The Development of Inhibitors of the SWI/SNF (BAF) Chromatin Remodeling Complex	83	Friday 19
David Earl	Biosynthetic Engineering of Apoptolidin and Ammocidin, Selective Inducers of Cell Death	84	Friday 20
Nora R. Eibergen	Development of Chemical Tools to Characterize the <i>Pseudomonas aeruginosa</i> Quorum Sensing Circuit	85	Friday 21
Aubrey J. Ellison	Collagen Mimetic Peptides for Probing Bacterial Infection	86	Friday 22
Graham S. Erwin	Mapping Polyamide–DNA Interactions in Human Cells Reveals a New Design Strategy for Effective Targeting of Genomic Site	87	Friday 23
Joshua M. Fishman	Polyoxazinones: The First Degradable and Functionalizable ROMP Polymer	88	Friday 24
Peter K. Foote	Towards Selective Inhibitors of Ubiquitin-Activating E1 Enzymes	89	Friday 25
Bennett Fox	Proline-Rich Repeat Domain Modulates Ubiquitin-Binding and Protein Stability of Ubiquilin 2	90	Friday 26
Amy Funk	Elucidation of the Isoprenylated Substrate Binding Site in Ras Carboxyl Methyltransferase using Biotinylated Photoaffinity Probes	91	Friday 27
Chantal Garcia De Gonzalo	NMR Structure and Mode of Action Studies of the S-linked Glycopeptide Sublancin	92	Friday 28
Clifford T. Gee	Developing Protein Observed ¹⁹ F NMR for Fragment-Based Screening to Target Protein-Protein Interactions	93	Friday 29
Joseph P. Gerdt	Mutational Analysis of the Quorum-Sensing Receptor LasR Reveals Interactions That Govern Activation and Inhibition by Non-Native Ligands	94	Friday 30
Eve A. Granatosky	Evaluation of the Polyketide GEX1A as a Potential Lead for Niemann-Pick Type C Disease	95	Friday 31
Joseph C. Grim	Noncarbohydrate Glycomimetics as Probes of C-type Lectin Function	96	Friday 32

Poster Presenter Index

Presenter	Poster Title	Abstract Page	Board Number
Marlies V. Hager	Development of α/β Peptides to Mimic Glucagon-like Peptide 1 (GLP-1)	97	Friday 33
Douglas A. Hansen	Development of in vitro Polyketide Synthase Catalysis and Evaluation of Substrate Promiscuity	98	Friday 34
Sarah Hansen	Reconstitution and Single Molecule Characterization of Yeast Spliceosomal Commitment Complex	99	Friday 35
Zvi Hayouka	Towards the Design of Effective Antibacterial Agents	100	Friday 36
Kevin Hwang	Caged DNazymes for Cellular Metal Ion Sensing	101	Friday 37
Jiaoyang Jiang	O-GlcNAc Transferase Makes the Cut	102	Friday 38
Andrew R. Johnson	An Untargeted Metabolomics Approach for Discovery of Novel Natural Products from Genetically-Perturbed <i>Streptomyces</i>	103	Friday 39
Carrie Johnson	Chemical Biology Probes for The SUMO protease SENP1, a Novel Target for Prostate Cancer Therapy	104	Friday 40
Alexander Justen	Using GlfT2 Point Mutants to Interrogate Galactan Chain Length in <i>Mycobacterium smegmatis</i>	105	Friday 41
Stefan Kathman	Covalent Inhibitors of HECT E3 Nedd4-1 Processivity Discovered with Irreversible Tethering	106	Friday 42
Virginia A. Kincaid	Virtual Screening for Competitive UDP-galactopyranose Mutase Ligands Identifies Novel Class of Antimycobacterial Agents	107	Friday 43
Anna Jo Komor	New Roles for Oxygen-Activating Diiron Enzymes in Antibiotic Biosynthesis	108	Saturday 44
E. Nihal Korkmaz	Structural Characterization of Ubiquitin Trimers using Small Angle X-Ray Scattering and Molecular Dynamics Simulations	109	Saturday 45
Kyle J. Korshavn	A Small Molecule That Displays Marked Reactivity Toward Copper- <i>versus</i> Zinc Amyloid- β Implicated in Alzheimer's Disease	110	Saturday 46
David T. Krist	A New Binding Site on E6AP for UbcH7	111-112	Saturday 47
Casey J. Krusemark	Functional Reverse Translation Via DNA-encoded Proteomic Probes	113	Saturday 48
Jean M. Lodge	Covalent Small Molecule Modulators of Coactivator-Activator Interactions	114	Saturday 49

Presenter	Poster Title	Abstract Page	Board Number
John C. Lukesh, III	A Potent, Versatile Disulfide-Reducing Agent from Aspartic Acid	115	Saturday 50
Mohammad M. Mahmoodi	Nitrodibenzofuran: an Efficient One- and Two-Photon Sensitive Photoremovable Protecting Group for Thiol Protection in Peptides	116	Saturday 51
Kimberly M. Maize	Human Hints: Powerful Tools for Nucleotide Monophosphate Drug Activation	117	Saturday 52
Sayaka Masuko	Elucidating the Roles of Glycosaminoglycans in Human Pluripotent Stem Cell Self-renewal and Differentiation	118	Saturday 53
Tucker Maxson	HIV Protease Inhibitors as Probes of Virulence Factor Production and Function	119	Saturday 54
Michelle M. Mitchener	Competition Between Arachidonic Acid and Endocannabinoids for Oxygenation by Cyclooxygenase-2	120	Saturday 55
Kalie A. Mix	Diazo Compounds for Bioversible Esterification of Proteins	121	Saturday 56
Joseph D. Moore	Active Efflux Influences the Activity of Quorum Sensing Inhibitors in <i>Pseudomonas aeruginosa</i>	122	Saturday 57
Corey Nemec	RNA Polymerase II C-terminal Domain Phosphorylation Mediates the Environmental Stress Response in Yeast	123	Saturday 58
Robert W. Newberry	Electronic Effects on Biomolecular Structure	124	Saturday 59
Sungjin Park	Protein Ubiquitination by HECT E3s without ATP, E1 and E2 Enzymes	125-126	Saturday 60
Katie L. Peterson	Effect of Lanthanide Complex Structure on Cell Viability and Association	127	Saturday 61
Grace H. Pham	Conformational Dynamics of Ubiquitin Signaling	128	Saturday 62
William C. Pomerantz	Aromatic Amino Acids: Privileged Side-Chains For Protein-Protein Interaction Inhibitor Discovery	129	Saturday 63
Rachel Pricer	Capturing the <i>In Vivo</i> Binding Partners of Transcriptional Activators Using a Genetically Incorporated Photocrosslinking Amino Acid	130	Saturday 64
Susan Ramos-Hunter	Investigation of G-protein Activated Inward Rectifying Potassium Channel Activator Lead to the Development of Specialized Photo Affinity Scaffolds for Structure Activity Relationship	131	Saturday 65
Andrew G. Reidenbach	Structure of Mitochondrial ADCK3 Defines an Ancient Protein Kinase-like Fold	132	Saturday 66

Poster Presenter Index

Presenter	Poster Title	Abstract Page	Board Number
Heather M. Robison	Label-Free Assay for Classifying Intact Human Breast Cancer Cells Using Desorption Electrospray Ionization Mass Spectrometry	133	Saturday 67
David Rogawski	Small Molecule Inhibitors of the Ash1L Histone Methyltransferase	134	Saturday 68
Danialle J. Ronnow	Mutasyntesis of Apoptolidin Analogs	135	Saturday 69
Julia Schwartzman	'Negotiations' in Symbiosis: Periodic Provision of Host Chitin Drives Rhythms in Symbiont Metabolism	136	Saturday 70
Younghee Shin	Development of Helical Peptidomimetics: Heterogeneous Peptide Backbones with α - and Preorganized β - and γ - Amino Acids	137	Saturday 71
Brian C. Smith	Nitric Oxide Signaling through <i>S</i> -nitrosoglutathione Transnitrosation	138	Saturday 72
Thomas P. Smith	Biomedical Applications and Strategies Utilizing Boronic Acid	139	Saturday 73
James D. Vasta	Development of Selective and Bioavailable Inhibitors of Collagen Prolyl 4-Hydroxylase	140	Saturday 74
Kyle D. Watson	The Development of Metal-Mediated Methodologies for the Syntheses of Potential Antibiotics	141	Saturday 75
Anna E. Weber	Investigation and Characterization of Scanning Ion Conductance Microscopy Resolution	142	Saturday 76
Michael A. Welsh	Small Molecule Modulation of RhIR Activity Induces Inverse Regulation of Pyocyanin and Rhamnolipid Production In <i>Pseudomonas aeruginosa</i>	143	Saturday 77
Marta Wenzler	Identification of Potential Circadian Rhythm Modulators	144	Saturday 78
Darryl A. Wesener	Recognition of Non-Mammalian Glycan Epitopes by Human Intelectin	145	Saturday 79
Paul J. Wrighton	Modular Surfaces Deliver Insoluble Cues to Promote Mesendoderm Differentiation of Human Pluripotent Stem Cells	146	Saturday 80
Yefim Zaltsman	The Role of Substrate Elasticity in Influencing the Differentiation of Human Pluripotent Stem Cells to Neurons	147	Saturday 81
Yi Zhang	Simultaneous Site-specific Dual Protein Labeling Using Multiple Protein Prenyltransferases	148	Saturday 82
Daniel B. Zwick	Synthetic Antigens and Calcium Flux Imaging Reveal Mechanisms of B cell-T cell Cooperation	149	Saturday 83

Development of Activity-Based Probes for Ubiquitin and Ubiquitin-Like Protein Signaling Pathways

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Ubiquitin and ubiquitin-like proteins (UBLs) diversify protein functions via reversible posttranslational modification of proteins. Consequently, misregulation of the UBL systems results in serious pathological disorders such as cancers, neurodegenerative disorders, and cardiovascular diseases.¹ However, our understanding of distinctive roles of ubiquitin and UBL pathways is still in its infancy. Therefore, deeper understanding of how UBL systems work, how they affect certain diseases and what other biological systems are associated with them will significantly contribute to curing human diseases.

Central to UBL protein signaling pathways are UBL protein-activating E1 enzymes that activate the C-terminus of UBL proteins for subsequent conjugation to the protein substrates. Due to their critical roles in biology, E1 enzymes have been recognized as emerging drug targets to treat human diseases.² In spite of their biological significance, however, methods to monitor the intracellular activity of E1 enzymes are lacking. Also, given that E1 enzymes are emerging drug targets, there is a critical need for methods to evaluate the intracellular mechanisms of action of E1 enzyme inhibitors.

We began to address these issues by developing a mechanism-based small molecule probe **ABP1** that can covalently label UBL proteins in the E1 activity dependent manner (Figure 1).³ Subsequently formed UBL-**ABP1** covalent adducts were then visualized and isolated via a click chemistry protocol. We demonstrated that **ABP1** could be used to monitor the global activity of E1 enzymes inside intact cells. The developed probe can also be used to profile the selectivity of E1 enzyme-targeting drugs *in vitro* and inside intact cells.

As a future direction, we propose rational strategies to design **ABP1** analogues that can have improved efficiency and selectivity in labeling UBL proteins. We expect that the proposed **ABP1** analogues will serve as a powerful tool to discover new ubiquitin-like proteins in human. In addition, the probes will provide a starting point for developing selective inhibitors for E1 enzyme family.

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Development of Activity-Based Probes for Ubiquitin and Ubiquitin-Like Protein Signaling Pathways

Heeseon An* and Alexander V. Statsyuk
continued

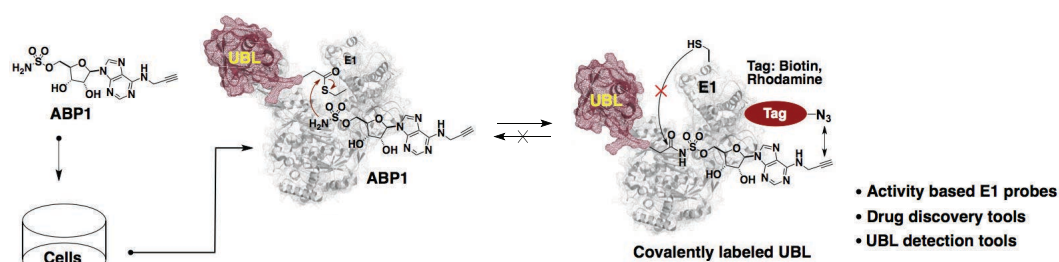


Figure 1. Design and applications of ABP1

“Early Drug Discovery Services at UW-Madison Small Molecule Screening and Synthesis Facility”

Gene E. Ananiev*, John Feltenberger, Ken A. Satyshur, Song Guo, Keith Behrend, Michael Hoffmann

UWCCC Small Molecule Screening and Synthesis Facility, University of Wisconsin Madison

Established in 2003, the Small Molecule Screening and Synthesis Facility (SMSSF) uniquely provides early drug discovery services to UW researchers in three core areas: (1) high throughput screening (HTS), (2) medicinal chemistry, and (3) computational chemistry. Located within the UW Carbone Cancer Center, SMSSF scientists work with individual UW researchers to provide expertise and infrastructure to support research projects. Staff scientists identify specific chemicals from the chemical libraries on hand that have unique functions relevant to the research of the specific investigator and provide training to UW students and postdoctoral scientists. The facility scientists also develop and implement mechanism of action assays that are provided to chemists bringing new compounds or libraries of compounds to the SMSSF for analysis. Optimization of compounds is supported through the SMSSF medicinal chemistry and molecular modeling services. Here we will present several examples of research projects conducted at the SMSSF. Additionally, a detailed description of our chemical libraries, liquid handling, synthesis and virtual screening capabilities will be presented.

Differential Labeling of Cell Surface Glycans with Diazo and Azido Sugars

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Understanding of glycan biosynthesis remains elusive due to the complexity of the number of precursors as well as the heterogeneity of polysaccharide products. In recent years, the incorporation of unnatural functional groups into the monomeric sugar building blocks has elucidated many aspects of glycan biosynthesis. These functionalities must be small enough to be tolerated by the series of enzymes that handle these sugars, and must also be bioorthogonal. One such functional group is the azido group (N_3), which can react with strained cyclooctynes in a 1,3-dipolar cycloaddition. The azide has been shown to be tolerated in several precursor sugars at specific positions that are untouched during metabolic processing. Here, we have developed a second functional handle, the diazo group (N_2), which is similar in size to the azido group, but possesses additional reactivity. The diazo-substituted *N*-acetylmannosamine is trafficked similarly to its azide counterpart and is expressed on the cell surface. In these preliminary experiments, both the diazo and azido-containing glycans were labeled with aza-dibenzocyclooctyne (DIBAC). Our goal is now to develop a unique reaction pair for the selective labeling of the diazo over the azide, taking advantage of the more extensive reactivity of the diazo group as determined by in vitro testing. Ultimately, these chemoselective reaction pairs can be utilized for dual metabolic labeling in living cells to expand the knowledge of glycan biosynthesis.

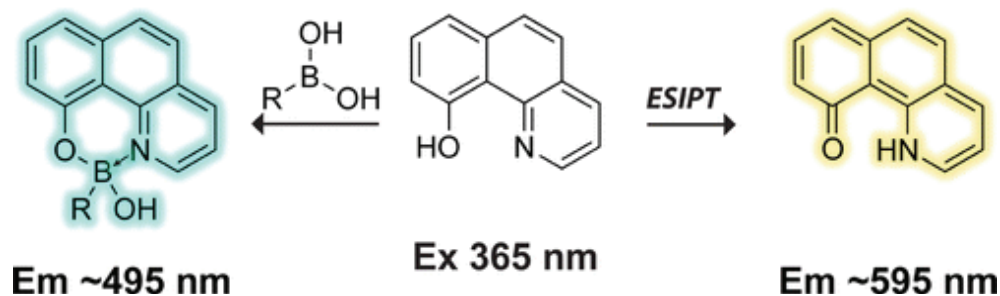
Protection and Detection of Boronic Acids for Chemical Biology

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The application of boronic acids cannot be overstated. These compounds are ubiquitous in synthetic labs as cross-coupling reagents, and lately show potential as valuable pharmacophores of interest to the chemical biology community. Despite their broad applicability, there are still difficulties in assessing them during reaction and purification. Hence, a general method for the rapid detection of boronic acids can have significant value to the chemical community. Our method allows for selective identification boronic acids during the reaction and purification steps. We apply an ESIPT photophysical process that provides a distinct “turn-on” fluorescent signal in the presence of boronic acids.

Benzoxaborole is an annulated boronic acid that—in the last five years—has become a privileged structure in drug discovery and biotechnology. Well beyond its role as “just another” reacting partner in Suzuki–Miyaura reactions, the benzoxaborole scaffold can serve as a potent pharmacophore in medicinal chemistry and possesses desirable properties for carbohydrate recognition at physiological pH. Its empty boron *p*-orbital, however, complicates its reactivity and isolation. Classical tri-coordinate boron-protecting groups are ineffective for protection of benzoxaborole. We report on a novel protecting group designed to resolve current limitations. The resulting protected complexes are easily formed and readily compatible with extractive and chromatographic separations, as well as synthetic reagents commonly employed in multistep syntheses. The protecting group is stable to basic and anhydrous acidic conditions, but cleaves readily to return intact benzoxaborole with aqueous acid.



Influence of O₂ on the Ferric Uptake Regulator (Fur) Regulon in *Escherichia coli* K12

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Metals, such as Fe, are essential to cellular function as they are integral cofactors to many proteins. Maintaining the appropriate levels of Fe²⁺ in cells is challenging due to its scarcity in the environment and its ease of oxidation by O₂. We study the regulation of cellular Fe levels in *Escherichia coli* K12 by analyzing the function of the Fe sensing transcription factor Fur. Fur maintains cellular Fe homeostasis by regulating the expression of many classes of genes, including genes that encode iron transporters, iron storage complexes, and iron-containing proteins. When Fe²⁺ levels are sufficient, Fe²⁺-bound Fur binds promoter regions upstream of genes resulting in gene repression. During Fe limitation, Fe-less Fur dissociates from these promoters resulting in gene expression. Despite extensive studies of the function of Fur during aerobic growth, its role in maintaining Fe homeostasis under anaerobic growth conditions, which mimic the human intestine, are not well understood. I have mapped the *in vivo* binding sites for Fur under both aerobic and anaerobic growth conditions *via* chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) and completed microarray-based transcriptomic analyses of strains lacking Fur compared to wild-type under aerobic and anaerobic growth conditions. I found that anaerobiosis increased Fur binding throughout the genome and caused an increase in repression of many Fur target genes. Electron paramagnetic resonance (EPR) was used to examine the levels of Fe available for Fur to bind under aerobic and anaerobic growth conditions. Our detailed analysis revealed that Fur function diverges under aerobic and anaerobic growth conditions to provide the cell with the necessary gene products to respond to dissimilar cellular iron demands and O₂-induced stresses.

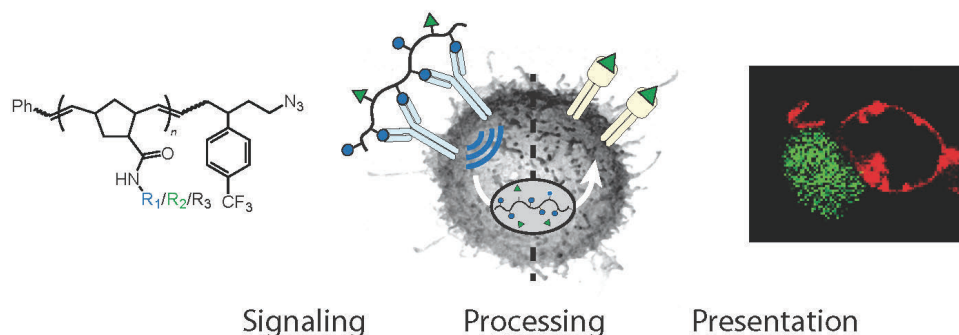
Dissecting Immune Signaling with Synthetic Multivalent Ligands

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Synthetic antigens from ROMP



B cells become activated towards humoral immunity through integration of two signals: antigen binding to the B cell receptor (BCR) and the recruitment of CD4⁺ T cells. The BCR plays a complex role in this process; it mediates both signal transduction in response to antigen binding and presentation of antigen on MHC class II molecules (MHCII). Identifying molecular interactions between antigen and the BCR that promote robust signal transduction and antigen presentation is essential for designing synthetic vaccines. Previously, we have shown that multivalent BCR ligand derived from ring-opening metathesis polymerization (ROMP) behaves as synthetic antigen: effectively clustering the BCR and activating B cell signaling.¹ By systematically altering ligand parameters, BCR signal output can be tuned.¹⁻³ To capitalize and extend these results, we envisioned using a polymer scaffold bearing peptide cargo that can be used to deliver antigen for presentation. Such a material could be capable of inducing humoral immunity. To this end, we synthesized a polymer functionalized with multivalent BCR ligand and T cell epitope. This compound is capable of activating signaling through the BCR, enabling cellular processing to release peptide cargo, and presenting peptide-MHCII for activation of CD4⁺ T cells. Our data validates an *in cellulo* system where antigen parameters can be systematically altered and its effect on activation of both B and T cells can be measured quantitatively. Such a system will provide insight for designing synthetic vaccines that maximize the humoral immune response.

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Small-molecule priming of procaspase-3 lowers the apoptotic threshold of cancer cells

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Combination therapy is the foundation of the clinical management of cancer. Typically combinations are discovered ad hoc and consist of drugs that target different biochemical pathways or those that act on different targets in the same pathway. Herein we demonstrate the utility of procaspase-3 targeted combination therapy. When procaspase-3 is treated with two activators, functioning by different mechanisms, considerable synergy is achieved; activating procaspase-3 in vitro, stimulating rapid and dramatic maturation of procaspase-3 in multiple cancer cell lines, and powerfully inducing caspase-dependent apoptotic death. In addition, the combination effectively reduces tumor burden in a murine lymphoma model at dosages for which the compounds alone have minimal or no effect. These data suggest the potential of procaspase-3 targeted combinations for the treatment of cancer and, more broadly, demonstrate that differentially acting enzyme activators can potently synergize to give a significantly heightened biological effect.

Elucidating the Mechanisms by which Non-native Acylated L-Homoserine Lactones Modulate LuxR-type Quorum Sensing Receptors in Bacteria

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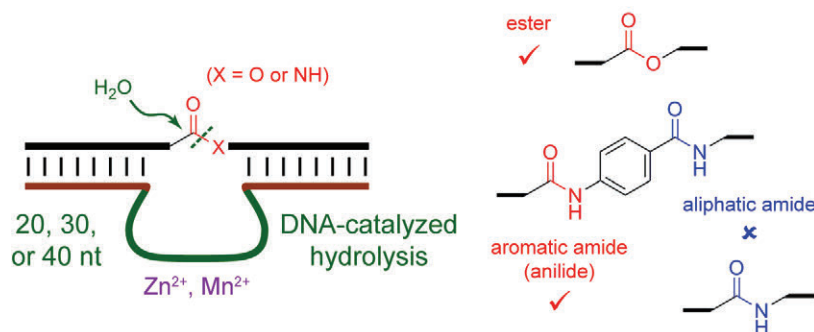
Quorum sensing (QS) is a widespread cell-to-cell communication system based on chemical signals that bacteria use to gauge colony size and modify their collective behaviors to benefit the group. Gram-negative bacteria use *N*-acylated L-homoserine lactone (AHL) signals for QS. The AHLs bind to intracellular LuxR-type receptors, which in turn dimerize, bind to the DNA, and transcribe group-beneficial genes. The Blackwell group has identified a range of synthetic AHL analogs that strongly modulate LuxR-type receptors QscR and LasR in *Pseudomonas aeruginosa*, an opportunistic pathogen that has developed resistance to traditional antibiotics. However, these non-native AHLs require further structural optimization to increase their potency, solubility, and physical stability. Based on recent biochemical studies of QscR and LasR, a non-native AHL is thought to displace a native ligand during protein folding, dimerization, or DNA binding. We therefore hypothesize that non-native AHLs will interact with the ligand-binding domains of LuxR-type receptors and antagonists will operate either by destabilizing the protein, preventing dimerization, or preventing DNA binding depending on the non-native ligand type. This study will detail the work thus far in characterizing non-native AHL modes of action. Teasing out mechanistic details by which non-native AHLs modulate LuxR-type receptors will allow for further refinement of non-native AHL structures for QS modulation, permitting their usage in a range of biologically relevant contexts and possibly paving a route to novel therapeutic development.

DNA-Catalyzed Hydrolysis of Esters and Aromatic Amides

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We previously reported that DNA catalysts (deoxyribozymes) can hydrolyze DNA phosphodiester linkages, but DNA-catalyzed amide bond hydrolysis has been elusive, despite having a much shorter uncatalyzed half-life of hydrolysis compared to a phosphodiester bond. Here we used in vitro selection to identify DNA catalysts that hydrolyze ester linkages as well as DNA catalysts that hydrolyze aromatic amides, for which the leaving group is an aniline moiety. The aromatic amide-hydrolyzing deoxyribozymes were examined using linear free energy relationship analysis. The hydrolysis reaction is unaffected by electron-donating or electron-withdrawing substituents on the aromatic ring, suggesting general acid-catalyzed elimination as the likely rate-determining step of the addition-elimination hydrolysis mechanism. These findings establish that DNA has the catalytic ability to achieve hydrolysis of esters and aromatic amides as carbonyl-based substrates, and they suggest a mechanism-based approach to achieve DNA-catalyzed aliphatic amide hydrolysis.



Chemical Tools to Study and Exploit Bacterial Cell Wall Assembly

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Pathogenic species of mycobacteria and corynebacteria possess a thick and impermeable cell wall that helps them evade detection and treatment by denying drug and probe molecules access to their cytoplasm. A distinctive feature of this barrier is an additional hydrophobic outer membrane composed of mycolic acids, which are covalently bound to an arabinogalactan (AG) polysaccharide that provides a link to the cell surface peptidoglycan. Efforts to identify weaknesses in these outer defenses would benefit greatly from a more complete understanding of how its components are assembled and maintained throughout the life cycle of the organisms. To this end, we aim to develop chemical tools that specifically target and exploit the extracellular mycolyl transferase enzymes Ag85A–C, which mediate the covalent attachment of mycolic acids to the cell wall AG. We have designed compounds that we anticipate will be processed by Ag85 to covalently alter the cell wall. We envisage that such a chemical tool would facilitate the early detection of pathogenic mycobacteria, and allow us to study cell wall biogenesis throughout the life cycle of the organisms.

Chemical complementation of corynebacterial arabinan in a deficient mutant

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The genera *Mycobacteria* and *Corynebacteria* contain several important human pathogens, including *C. diphtheria* and *M. tuberculosis*, the latter of which still represents a leading cause of mortality in the developing world. The high virulence of *M. tuberculosis* is due in part to a thick, complex cell wall that shields the bacterium from drugs and other organisms. However, the role of cell wall may not be limited to protection because it is essential for viability. As a result, several important antimycobacterial drugs, such as ethambutol and isoniazid, target various aspects of cell wall biosynthesis.

The mycobacterial cell wall is composed of three domains: the peptidoglycan, the arabinogalactan, and the outer mycolic acid-rich lipid bilayer. The peptidoglycan resembles that of other gram-positive bacteria. However, extending from the peptidoglycan is the arabinogalactan polysaccharide that is capped at its termini with mycolic acids, the presence of which is unique to *Corynebacteria* and *Mycobacteria*. The arabinogalactan is composed of two sub-domains, the galactan, which is a linear polymer of galactofuranose, and the arabinan, a highly branched polymer of arabinofuranose. Assembly of the arabinan is performed by a series of arabinofuranosyl transferases that extend the growing polymer using decaprenyl phosphoryl arabinose (DPA) as their sugar donor. Although the structure of the arabinan is known, its biosynthesis and how branching affects cell wall integrity are less well understood.

We are interested in elucidating the role of the arabinan, and how the assembly of this complex polysaccharide is controlled. The work presented here demonstrates the ability of the arabinan to be recovered by the addition of DPA analogs to cultures of a *C. glutamicum* mutant that is unable to synthesize DPA. Using this model system we have probed what structural features of DPA are necessary for arabinose incorporation. Moreover, we determined the effect of complementation on cell morphology. Increasing our knowledge on these topics will expand our basic understanding of polysaccharides and potentially lead to the discovery of novel therapeutic targets.

References:

Synthesis of Lipid-Linked Arabinofuranose Donors for Glycosyltransferases

Matthew B. Kraft, Mario A. Martinez Farias, and Laura L. Kiessling
The Journal of Organic Chemistry **2013** 78 (5), 2128-2133

Designing Parathyroid Hormone Mimics with Enhanced Properties via Sequence-Based α/β -Peptide Design

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Peptide-based therapeutics are a useful and valuable class of pharmaceutical agents. In 2011, there were over 40 approved peptide drugs on the market, totaling over \$14 billion in sales. Peptide-based agents often exhibit potent biological activities, good toxicological profiles and low levels of immunogenicity; however, they typically suffer from short half-lives *in vivo* and require administration by injection due in-part to degradation by endogenous proteases. One approach to counteract proteolytic instability relies on the incorporation of non-natural amino acid homologs, such as β -amino acid residues, that are not recognized by proteases. Peptides consisting of both α - and β -amino acids (α/β -peptides) show good proteolytic stability and have been effectively used to mimic natural peptides comprised entirely of α -amino acids.

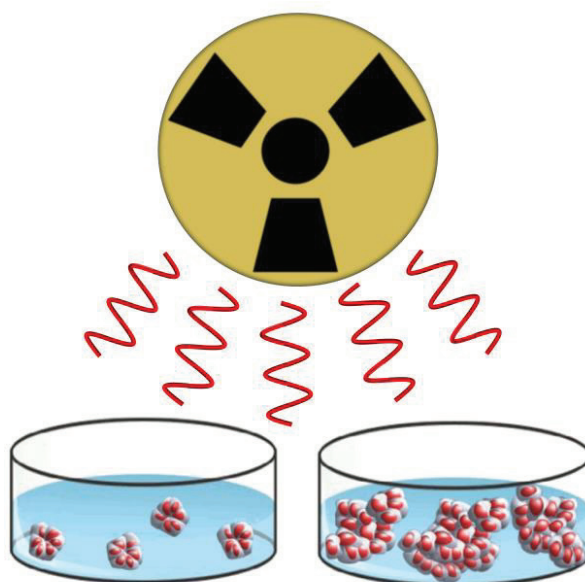
This research aims to design functional α/β -peptides that target the parathyroid hormone receptor (PTH1R), exhibit enhanced proteolytic stability and demonstrate interesting pharmacological properties. PTH1R represents an attractive target for these studies as once-daily subcutaneous injection of an N-terminal fragment of parathyroid hormone [PTH(1-34)] has been shown to be effective in stimulating bone growth and is used clinically for treating osteoporosis. Presented results demonstrate that α/β -peptide mimics of parathyroid hormone retain biological activity comparable to natural parathyroid hormone while showcasing enhanced protease stability *in vitro* and prolonged bioavailability *in vivo*. Selected α/β -peptide mimics of PTH demonstrate receptor conformation dependent binding profiles that diverge from that of natural PTH, which is manifested in animal studies as variations in the duration of biological responses observed. Application of the approaches developed in these studies may support development of PTH-based therapeutics with fewer side effects that can be dosed via less frequent injections or administered orally.

"Breaking Collagen": Regulating *In Vitro* HepG2 Malignancy with *In Situ* MMP Degradation of Collagen-PEG Hydrogels

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In vitro three dimensional (3D) tumor models are important tools for understanding cancer progression and evaluating new cancer treatments on a benchtop scale. Countless *in vivo* studies have demonstrated that cancer malignancy is based on extracellular matrix (ECM) remodeling. However, few 3D tumor models have been able to replicate these ECM changes *in vitro*. Therefore, this study demonstrates a new strategy to induce *in vitro* matrix degradation using a matrix metalloproteinase (MMP) often overexpressed by cells in the tumor microenvironment. With this approach, we explored the effects of selective matrix degradation on hepatocarcinoma malignancy and radiosensitivity. In this study, we prepared a collagen gel cross-linked by polyethylene glycol disuccinimidylester, in which hepatocarcinoma cells exhibited a benign, hepatocyte-like phenotype. However, exposing this gel to MMP decreased its elastic modulus from 4.0 to 0.5 kPa, causing the hepatoid-like spheroids to revert to a malignant phenotype. Interestingly, the resulting malignant cancer cells were more sensitive to radiation than cells cultured in a stiff, non-degraded gel. Overall, our 3D tumor model will improve fundamental and applied cancer studies.



Radiosensitivity increased with increasing MMP-induced degradation

Metabolomics for Antibiotic Discovery and Mechanistic Characterization

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More than 85 years after Alexander Flemming's serendipitous discovery of penicillin, the mechanisms by which antibiotics lead to cell death are still debated, the antibiotic pipeline has nearly dried up, and antibiotic resistant pathogens dominate our hospitals. These seemingly insurmountable challenges in the fight against infectious disease have caused many to fear modern medicine is moving into a post-antibiotic era. However, using metabolomics analyses, we may be able to access a hidden antibiotic vault and elucidate the underlying mechanisms by which antibiotics kill microorganisms. The metabolome is the end point of the central dogma and thereby contains valuable information representing the phenotype of a cell. When exogenous compounds like antibiotics perturb a biological system, they alter the metabolic profile in a manner specific to the mechanism of action of the antibiotic. This report addresses the problems of novel antibiotic mining and discovery, and the subsequent elucidation of mechanism by employing metabolomics methods to determine the consequences of antibiosis and various derepression techniques to unlock a new antibiotic vault.

Acyl protein thioesterase 2 regulates MAPK/ERK signaling and cell polarity

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Loss of growth and polarity checkpoints are both critical steps in the initiation and progression of cancer. The MAPK/ERK transduction cascade coordinates a variety of cellular processes that are involved in epithelial-to-mesenchymal transition (EMT). Importantly, protein palmitoylation contributes to the membrane association, subcellular trafficking, stability and function of numerous regulatory proteins with key roles in cell growth, polarity, and signaling pathways. Recent reports demonstrate that acyl protein thioesterase (APT) inhibition leads to a reduction in MAPK/ERK signaling and partial phenotypic reversion of HRasG12V-induced EMT. Here we investigate the mechanism by which APTs perturb MAPK/ERK signaling and define a new role for protein palmitoylation in organizing cell junctions. We recently reported a class of *in vivo* active, small selective inhibitors of the acyl protein thioesterases APT1 and APT2. Addition of an APT2 inhibitor silences MAPK/ERK signaling in a Snail-induced EMT cell line. Using bioorthogonal metabolic labeling methods, we find that Ras acylation levels are unaffected by APT inhibition. In contrast, we identify the tumor suppressor Scribble (Scrib) as a dynamic, APT2-regulated palmitoylated protein in malignant cells. Scrib localizes at the basolateral membrane where it regulates epithelial cell apical-basolateral polarity, junctional integrity, proliferation, and metastasis. Loss of Scrib at the plasma membrane is reported to cooperate with oncogenic Ras to drive tumor formation, promote EMT, and bypass contact inhibition. Notably, APT2 inhibition not only restores Scrib membrane localization, but also enhances expression of E-Cadherin (ECad), a central component of adherens junction necessary for establishing proper apical-basal polarity. These data are corroborated by recent reports of Scrib-mediated suppression of the MAPK cascade downstream of Ras, but upstream of ERK, a known activator of Snail-mediated silencing of Ecad. These findings suggest that thioesterases regulate the palmitoylation of cell polarity proteins, and highlight a strategy to attenuate oncogenic signaling and restore cell polarity pathways in malignant cells.

Functional Studies of Ebola Virus Matrix Protein VP40

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Filoviruses are filamentous viruses and include Ebola (EBOV) and Marburg (MARV), which are morphologically identical but antigenically distinct. These remarkable viruses can vary in length from ~1 to 14 μm and are pleomorphic in shape. Mortality rates can be as high as 90% and to date there are no FDA approved vaccines or small molecules for treatment. EBOV harbors a genome of 7 proteins, the most abundantly expressed of which is Viral Protein 40 (VP40) also known as the matrix protein. VP40 is required for the assembly and budding of EBOV and alone VP40 can form virus like particles (VLPs) from the plasma membrane of host cells. Recent work by the Stahelin and Ollmann-Saphire (Scripps Research Institute) labs indicates that VP40 adopts two different structures to elicit different functions in the viral life cycle. Cellular data demonstrates that each structure adopts a specific function, one for budding from the plasma membrane of human cells and one for regulation of viral transcription. This work investigates how distinct VP40 structures assemble in the presence of synthetic lipid vesicles and at the inner leaflet of the plasma membrane in live cells. This project aims to determine lipid composition requirements for functionality of VP40 mutants vs. wild-type VP40, and to elucidate the function of VP40 oligomerization with site-specific mutants.

Preliminary data suggest that VP40 binds with nanomolar affinity to phosphatidylserine (PS), an anionic lipid found within the inner leaflet of the plasma membrane. Cellular data from the Stahelin lab provides precedent that VP40 requires PS in order to bud from the plasma membrane. PS-depleted cells show diminished plasma membrane budding that is subsequently rescued upon supplementation of PS. Probing other lipids with mutant forms of VP40 have helped identify regions of the protein that are integral in PS binding. Large Unilamellar Vesicle (LUV) assays have indicated that VP40 binds some, but not all, phosphatidylinositol (PIP) family members, indicating that the VP40-PIP interaction is not merely a charge-sensing interaction. ITC data on these PIP family members also suggests lipid headgroup specificity.

This work is supported by NIH grant AI081077.

Investigating Sterol-Amphotericin B Interactions by Solid-State NMR Spectroscopy

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Amphotericin B (AmB) is a powerful antifungal drug of last resort to treat life threatening fungal infections, used for over fifty years with close to zero clinically relevant microbial resistances. We have recently determined that AmB kills yeast cells primarily by binding to ergosterol¹ and forming a large extramembranous sterol sponge.² This model hypothesizes that interactions of AmB with ergosterol determine its ability to kill yeast, whereas binding to cholesterol is responsible for determining its toxicity in human cells. Solid-state NMR (SSNMR) spectroscopy is uniquely able to detect and quantify the binding of sterols to AmB in the sterol sponge at atomistic detail. Using a variety of SSNMR methods, we aim to understand the key interactions that determine the sterol-specificity of AmB, which will provide an avenue to an improved therapeutic index potentially leading to new analogs of AmB that are toxic to fungal cells but not to humans.

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Nickel Superoxide Dismutase: Spectroscopic and Computational Evaluation of the Roles Played by the Secondary and Tertiary Sphere Residues Glu17 and Arg47

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Nickel-dependent superoxide dismutase (NiSOD) is the most recently discovered member of the family of metalloenzymes that regulate the buildup of the superoxide anion ($O_2^{\bullet -}$) and other reactive oxygen species (ROS)¹. In catalyzing the disproportionation reaction of superoxide, the reduced Ni center converts from a square-planar, four-coordinate Ni^{2+} state to a square pyramidal, five-coordinate Ni^{3+} state via axial coordination of a nitrogen (N_{ax}) from the imidazole of His1. Crystallographic data² has revealed that the Ni– N_{ax} bond is unusually long (~ 2.3 Å) when compared with typical Ni–nitrogen bond distances (~ 2.0 Å). This difference has been attributed to the existence of a hydrogen-bonding network involving the secondary and tertiary sphere residues Glu17 and Arg47, respectively, that is well positioned to potentially modulate the Ni– N_{ax} bond distance. To test this hypothesis, the R47A NiSOD variant was constructed in which the hydrogen-bonding network is sufficiently perturbed to cause a decrease in catalytic activity³. In the present study, we have performed a combined spectroscopic and computational investigation of the R47A NiSOD variant to assess the influence of the His–Glu–Arg hydrogen bonding network on the active-site properties. Small but noticeable differences are observed between the electronic absorption, circular dichroism (CD), and magnetic CD spectra of the oxidized states of the wild-type enzyme and the R47A NiSOD variant. Our computational results, supported by our experimental evidence, indicate that the R47A substitution causes an elongation of the Ni– N_{ax} bond, thereby leading to a stabilization of the Ni $3dz^2$ -based redox active molecular orbital. This change in electronic structure is expected to stabilize the Ni^{2+} state over the Ni^{3+} state, consistent with the decrease in catalytic turnover. These findings have important implications for the design of new five-coordinate NiSOD biomimetics.

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The Development of Inhibitors of the SWI/SNF (BAF) Chromatin Remodeling Complex

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Recent exome sequencing of human tumors has uncovered a surprising new role for chromatin regulation in the development of cancer. Of particular note is the frequent identification of mutations in one or more subunit of the SWI/SNF (or BAF) chromatin remodeling complex. For an increasing number of these cancers, mutation in one subunit produces a residual, oncogenic complex, suggesting that the BAF complex itself is a good therapeutic target. Several additional lines of data suggest that inhibition of chromatin remodeling complexes could be therapeutic; however no inhibitor of an ATP-dependent chromatin remodeler has yet been identified. To this end, we have performed a large, high throughput screen monitoring BAF mediated transcription. After further validation, we have identified a handful of compounds that regulate transcription in a manner similar to the BAF knockout. We have used this approach to identify topoisomerase inhibitors as potent regulators of chromatin remodeling. In addition we have identified several novel compounds that may be directly inhibiting the BAF complex.

In order to confirm that the novel compounds act directly on the BAF complex, we are synthesizing focused small molecule libraries for the optimization of target identification probes. While some of the compounds may not inhibit the BAF complex directly, deciphering their targets will give us great insight into the mechanism of BAF-mediated transcription, and possibly BAF-mediated tumor suppression. With a BAF inhibitor in hand, we will have the tools for understanding the tumor suppressive mechanism of the BAF complex, as well as confirming the therapeutic potential of targeting this class of epigenetic regulators in cancer.

Biosynthetic Engineering of Apoptolidin and Ammocidin, Selective Inducers of Cell Death

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Apoptolidin and ammocidin are selectively, cytotoxic macrolides produced by members of the gram-positive bacteria genus *Ammycolatopsis*. Remarkably, these compounds are low nanomolar inhibitors of melanomas and glioblastomas, two cancers for which no viable therapeutic interventions exist. Further these natural products do not inhibit proliferation of non-transformed cells. By combining biosynthetic and synthetic approaches, we have been able to determine critical structure activity relationships and have developed a suite of probe compounds to study the molecular underpinnings of this remarkable selectivity. Currently, we are studying the mode of cell death induced by apoptolidin and ammocidin and are undertaking a multi-pronged approach to identify the molecular target by chemical proteomics, gene knockout libraries, and evaluation of cell signaling node activation in primary tumor samples.

Development of Chemical Tools to Characterize the *Pseudomonas aeruginosa* Quorum Sensing Circuit

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Bacteria use quorum sensing (QS) to induce specific phenotypes as a function of population density. In Gram-negative bacteria, this QS process is largely driven by the detection of structurally distinct *N*-acylated L-homoserine lactones (AHLs). These bacteria produce AHLs that diffuse across the membrane, and at high cell densities, intracellular AHL concentrations are sufficient to bind cognate receptors, induce the formation of transcriptionally active receptor dimers, and thereby promote the transcription of target genes. Many of these target genes promote bacterial virulence; therefore, the disruption of bacterial QS with small molecules is an attractive antivirulence strategy. The QS system of *P. aeruginosa* is a particularly attractive target for this strategy, as this pathogen is prevalent in the lungs of cystic fibrosis patients, is resistant to most traditional antibiotics, and employs QS to regulate virulence factor production and biofilm formation. However, the *P. aeruginosa* QS system is also very complex, as it employs three receptors (LasR, RhlR, and QscR) and two AHLs. The activities of these three receptors are intimately interconnected, and one of the AHLs produced is recognized by two receptors. The complexity of this QS system has prevented its complete characterization and thereby hampered its exploitation as a target for antivirulence agents. Therefore, our efforts are aimed at obtaining a greater understanding of the receptor interactions that govern QS regulation of *P. aeruginosa* virulence. To this end, we plan to use non-native AHLs as chemical tools to probe the contributions of individual receptors on *P. aeruginosa* virulence and thereby facilitate the delineation of receptor hierarchy in this pathogen. Described herein are our efforts to identify non-native AHLs that modulate *P. aeruginosa* QS in a receptor-selective manner. To do this, we first constructed sets of reporter strains that differ only by the promoters that are transcriptionally fused to the reporter gene. These strains allowed us to circumvent problems reported in the literature that are associated with comparing data sets obtained using different screening conditions and strains. The data obtained from screening our library of >250 non-native AHLs against these highly similar strains are the first of their kind generated for *P. aeruginosa* QS machinery. From these screens, several AHLs were identified that modulate QS in *P. aeruginosa*, and their activities against the three individual receptors of *P. aeruginosa* have been profiled using reporter strains of *E. coli*. The conclusions drawn from this study will be instrumental to the characterization of the *P. aeruginosa* QS system and could thereby facilitate efforts to develop antivirulence strategies for this dangerous pathogen.

Collagen Mimetic Peptides for Probing Bacterial Infection

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Invasion of host tissue can be mediated by the interaction of bacterial surface proteins and the extracellular matrix (ECM).¹ Group A *Streptococcus* (GAS) displays cell-surface proteins that resemble human collagen.^{2–5} Collagen is the most abundant protein in humans and comprises a major structural component of the ECM. In wounds, collagen triple helices suffer damage. We hypothesize that the collagen-like strands of GAS form triple helices with denatured collagen in a wound bed. The ensuing adherence could be a basis for infection by GAS. To test this hypothesis, we have used recombinant DNA technology to produce two of the collagen-like strands, Scl 1 and Scl 2, in *Escherichia coli*. We are also synthesizing a “nest” of two tethered collagen mimetic peptides (CMPs). This nest is intended to mimic the denatured collagen in a wound bed. Finally, we are using fluorophore-labeled CMPs and ultimately our nest to detect collagen strands on the surface of live GAS cells. With these tools we hope to understand and, ultimately, antagonize infections by GAS. This work is being supported by Chemistry–Biology Interface Training Grant T32 GM008505 and grant R01 AR044276 (NIH).

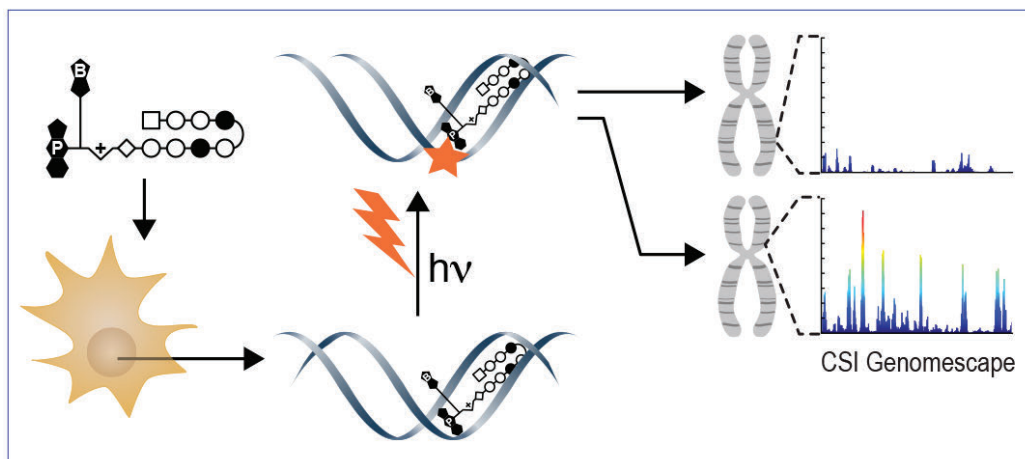
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Mapping polyamide–DNA interactions in human cells reveals a new design strategy for effective targeting of genomic sites

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Targeting the genome with sequence-specific synthetic molecules is a major goal at the interface of chemistry, biology, and personalized medicine. Pyrrole/imidazole based polyamides can be rationally designed to target specific DNA sequences with exquisite precision *in vitro*; however, the biological outcomes are often difficult to interpret using current models of binding energetics. To directly identify the binding sites of polyamides across the genome, we designed, synthesized, and tested polyamide derivatives that enabled covalent crosslinking and localization of polyamide–DNA interaction sites in live human cells. Bioinformatic analysis of the data reveals that clustered binding sites, spanning a broad range of affinities, best predict occupancy in cells. In contrast to the prevailing paradigm of targeting single high-affinity sites, our results point to a new design principle to deploy polyamides and perhaps other synthetic molecules to effectively target desired genomic sites *in vivo*.



Polyoxazinones: The First Degradable and Functionalizable ROMP Polymer

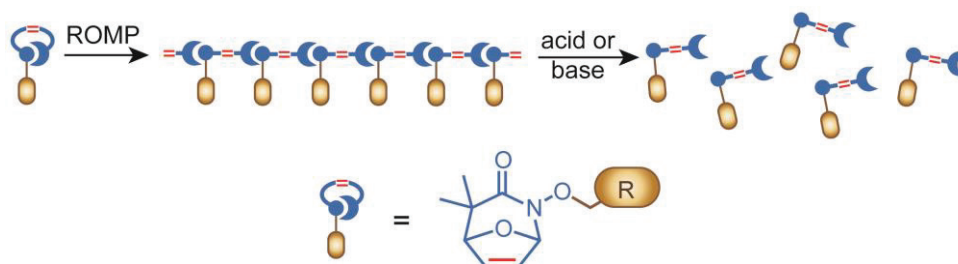
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A valuable approach for creating synthetic polymers is the ring-opening metathesis polymerization (ROMP). Well-defined metal carbene catalysts polymerize cyclic olefin monomers with high control over the polymer chain length and architecture. However, the utility of ROMP polymers with respect to structural tunability often sacrifices degradability. To address this issue, we recently identified a heterobicyclic olefin containing an oxazinone core as a new substrate for ROMP. The polymers produced undergo degradation when exposed to either acidic or basic conditions. Additionally, the monomers can be readily diversified to access degradable ROMP polymers bearing tailored functionality. Optimization of the polymerization of these monomers, as well as specific applications of the resultant polyoxazinone polymers, is discussed.



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Towards Selective Inhibitors of Ubiquitin-Activating E1 Enzymes

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In ubiquitin-like systems, each type of ubiquitin-like protein (UBL) is activated in an ATP-dependent manner by a specific, corresponding E1 enzyme, also called a ubiquitin-activating enzyme. The UBL is subsequently transferred to E2 and E3 enzymes for conjugation to a protein substrate. The position of E1 enzymes at the apex of these pathways makes them ripe targets for interrogating the function of each UBL. In particular, selective small-molecule inhibition of each E1 enzyme will allow the identification of UBL substrates and the deconvolution of UBL cross-regulation.

A major obstacle to the design of selective E1 inhibitors is the highly conserved nature of the ATP-binding E1 active site. A well-studied general E1 inhibitor scaffold is being used as a template for the design of a variety of E1 inhibitors¹. Current efforts have yielded inhibitors with modest potency both *in vitro* and in cellular assays against HCT-116 cells. We have hypothesized that subtle differences between selective and nonselective inhibitors may hold the key to our understanding of the E1 active sites of different ubiquitin-like proteins. Further synthetic optimization and SAR is under way to test these hypotheses.

Our initial focus has spanned both the Ubiquitin and Nedd8 E1 enzymes, but we hope to expand our methodology to other E1s in the future. Interrogation of UBL systems using selective E1 inhibitors will enable a more nuanced understanding of UBL protein function and validate these systems as pharmacological targets.

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Proline-Rich Repeat Domain Modulates Ubiquitin-Binding and Protein Stability of Ubiquilin 2

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The presence of intracellular aggregates containing misfolded and ubiquitin (Ub)-modified proteins is a hallmark of many neurodegenerative diseases, including Alzheimer's Disease, Parkinson's Disease, and amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease). In many cases, these aggregates also contain Ub-binding proteins, including members of the ubiquilin (UBQLN) family of molecular chaperones. UBQLN proteins function in Ub-mediated proteolysis as shuttling factors by interacting with both Ub-modified proteins and subunits of the proteasome. It was discovered in 2011 that missense mutations in the gene encoding *UBQLN2* cause dominantly inherited ALS and a mixed ALS/dementia phenotype. Disease-causing mutations in UBQLN2 are clustered in a proline-rich-repeat (PRR) domain not present in UBQLN1. In fact, UBQLN1 and UBQLN2 share 95% similarity and otherwise harbor the same type, number, and arrangement of modular domains – suggesting that the PRR may be of particular importance to UBQLN2 function. We discovered that UBQLN2 binds significantly more Ub than UBQLN1 in a PRR-dependent manner, as assessed by co-immunoprecipitation (IP) experiments employing both epitope-tagged and endogenous proteins. UBQLN2 proteins harboring disease-relevant mutations in the PRR exhibit an additional increase in Ub-binding affinity, observed by co-IP and *in vitro* experiments with recombinant proteins. This biochemical phenotype appears to be coupled to protein stability, such that greater Ub-binding affinity results in reduced protein half-life, as measured by semi-quantitative western blotting in an inducible cell culture model. These findings provide the first evidence of a functional distinction between UBQLN1 and UBQLN2, and suggest that mutations in the PRR cause hypermorphic Ub-binding. Collectively, these findings contribute to a better understanding of UBQLN function in protein degradation and disease.

Elucidation of the Isoprenylated Substrate Binding Site in Ras Carboxyl Methyltransferase using Biotinylated Photoaffinity Probes

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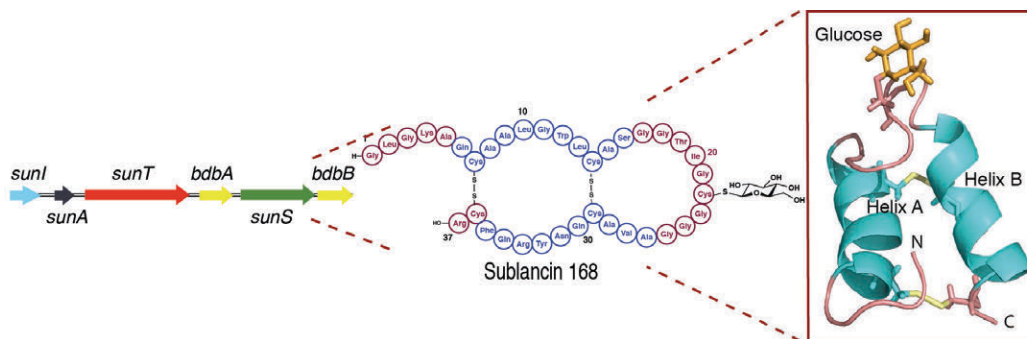
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CaaX proteins undergo three sequential post-translational modifications: isoprenylation of the cysteine residue, endoproteolysis of the –aaX residues and methylation of the isoprenylated cysteine by isoprenyl cysteine carboxyl methyltransferase (Icmt). We are focusing on an in-depth analysis of the structure and function of Ste14p, the Icmt from *S. cerevisiae*, as a model for the human enzyme. As methylation is critical for the proper localization of the CaaX protein K-Ras in humans and may be essential for oncogenic transformation, Icmt may prove to be an excellent chemotherapeutic target. We have generated purified and functionally reconstituted wild-type and Cys-less Ste14p along with a large library of cysteine-variants and other site-directed mutants. We are now poised to use these tools to understand in mechanistic detail how Ste14p mediates catalysis at the membrane/cytosol interface, accommodating both the hydrophilic co-factor *S*-adenosylmethionine (SAM) and a lipophilic isoprenylated protein substrate. To this end, we evaluated synthetic analogs of two well-characterized Ste14p substrates, N-acetyl-*S*-farnesyl-L-cysteine (AFC) and the yeast α -factor peptide mating pheromone, which contain photoactive benzophenone or diazirine moieties in either the lipid or peptide portion of the molecule. Both types of analogs were substrates for Ste14p. Photocrosslinking and neutravidin-agarose capture experiments with these analogs revealed that Ste14p was specifically photolabeled with all of the compounds tested. Using the cysteine-specific cleavage reagent NTCB, we demonstrated that AFC analog containing a benzophenone moiety directly upstream of the cysteine photolabeled residues in the first 77 amino acids of the protein. Together, our data suggest that these photoaffinity analogs will be useful for the future identification of the Icmt substrate binding site.

NMR Structure and Mode of Action Studies of the S-linked Glycopeptide Sublancin

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The rapid development of bacterial antibiotic resistance not only decreases the effectiveness of known antibiotics but also increases the need for the discovery of novel drugs. Ribosomally synthesized and post-translationally modified peptide (RiPP) natural products are a rapidly expanding class of such compounds. Sublancin 168, produced by *Bacillus subtilis* 168, belongs to the glycoцин family of RiPPs and contains a glucose moiety linked to a cysteine residue, an unprecedented post-translational modification assembled by a glycosyltransferase (SunS). Sublancin has been shown to be extremely stable and has a narrow spectrum of activity with an unknown mode of action. Its extreme stability and unique structure have led us to hypothesize that sublancin has a novel antimicrobial mechanism of action. The solution NMR structure of sublancin has been solved for clues regarding its mechanism of action and provides an explanation for the previously reported high stability of sublancin. Various biochemical, microbiological, and genomic tools have been employed to characterize sublancin's activity. Data obtained from comparative genomic analysis and global gene expression using DNA microarrays has identified the PTS-glucose specific transport system as a possible mechanism by which sublancin could affect the cell. Current efforts include investigating sublancin's localization in the cell by fluorescent sublancin analogues. In addition, we are synthesizing sublancin analogues with a phosphorylated glycan to investigate the role of the sugar. A clear understanding of how this unique antibiotic exerts its antimicrobial activity may facilitate the development of new antibiotics.



Developing Protein Observed ^{19}F NMR for Fragment-Based Screening to Target Protein-Protein Interactions

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This poster describes my recent work in performing the first demonstration of a full fragment screen using Protein Observed ^{19}F (PrOF) NMR targeting the KIX binding domain of the transcriptional coactivator CREB Binding Protein (CBP). Protein-protein interactions (PPIs) play a vital role in biological processes, making the ability to selectively target and modulate PPIs an important therapeutic challenge due to the links between dysregulated PPIs and disease.¹ While PPIs have long been thought to be undruggable, recent successes by Wells, Fesik, Vassilev, and others have shown the situation to be challenging but not impossible.² The central hypothesis of this project is that PrOF NMR can be used as a powerful fragment-based screening method to accomplish this goal. Fluorine is a useful NMR active nucleus due to its sensitivity to subtle changes in its chemical environment and its ability to rapidly acquire structural information regarding ligand binding or protein folding while overcoming challenges faced by other screening methods, such as superfluous background signals and probe insensitivity,³ making PrOF NMR an ideal fragment-based screening method.^{4,5}

Using PrOF NMR, the binding activity of small-molecules to the fluorinated protein is assessed by perturbations of the peaks in the protein's ^{19}F NMR spectrum. Thus far, I have screened 508 compounds, which has yielded 15 statistically relevant mixture hits. From deconvoluting those mixtures, I have identified several small-molecule ligands, evaluated the structure-activity relationship (SAR), and determined the binding affinities for KIX of four of the compounds. Ligand observed NMR has also been used as a secondary validation method. Further characterization of these compounds is ongoing.

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Mutational Analysis of the Quorum-Sensing Receptor LasR Reveals Interactions That Govern Activation and Inhibition by Non-Native Ligands

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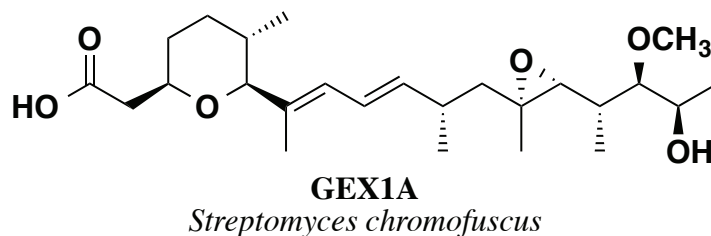
N-acylated-L-homoserine lactone (AHL) quorum sensing (QS) regulates the expression of pathogenic and mutualistic phenotypes in many bacteria. An improved molecular understanding of how chemical probes activate and inhibit QS should help to develop better experimental tools and potential therapeutics. We therefore mutated each hydrogen-bonding residue in the ligand-binding pocket of the *Pseudomonas aeruginosa* QS receptor LasR and used a reporter gene assay to test the activation and inhibition of these mutants by a range of non-native ligands. Our data show that the interactions between the ligands and Trp60, Tyr56, and Ser129 govern whether a ligand is an activator or inhibitor. Using this knowledge, we were able to hypothesize a cause for activation vs. inhibition of LasR—from a subtly different interaction with the binding pocket to a global change in LasR conformation.

Evaluation of the Polyketide GEX1A as a Potential Lead for Niemann-Pick Type C Disease

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Niemann-Pick Type C (NPC) disease is a rare neurodegenerative disorder in which cholesterol accumulates in the lysosomes as a result of a mutation in either the *NPC1* or *NPC2* gene. Recent studies have shown that histone deacetylase (HDAC) inhibitors are effective in reversing cholesterol accumulation in human NPC1 mutant fibroblasts. Our efforts to identify a novel treatment option for NPC disease are focused on the *Streptomyces*-derived polyketide GEX1A, which increases gene expression similar to known HDAC inhibitor trichostatin A. We have observed that GEX1A is capable of facilitating cholesterol trafficking in NPC1 cells, however GEX1A does not affect histone acetylation and likely acts through a novel mechanism. Based on these findings, we have developed a multidisciplinary route to accessing GEX1A, as well as synthetic, semisynthetic and bioengineered analogues, in order to investigate their potential as therapeutics for NPC disease. Here we highlight our approach to accessing GEX1A, describe our recent work in engineering mutant *Streptomyces chromofuscus* strains capable of generating GEX1A analogues, and present our current progress towards evaluating GEX1A in both NPC1 and NPC2 mutant fibroblasts.



Noncarbohydrate Glycomimetics as Probes of C-type Lectin Function

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C-type lectins receptors (CLRs) are a class of calcium-dependent carbohydrate binding proteins critical to many immunological processes. One CLR of particular interest, dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN), is co-opted by many pathogens for proliferation, but the mechanisms of these phenomena are poorly understood. Compounds that mimic pathogenic carbohydrate epitopes aid in understanding CLR function. Previously, we have generated D-mannose glycomimetics for DC-SIGN utilizing (-)-shikimic acid as a scaffold. However, for these compounds to truly be worthy of the term “glycomimetic” they must bind similarly to the endogenous carbohydrate and be capable of acting as an agonist of DC-SIGN. We first employed NMR to characterize their mechanism of binding to DC-SIGN and found the glycomimetics indeed bind similarly to D-mannose. As with all carbohydrate ligands, a multivalent display of glycomimetics enhances affinity and specificity. Protein-based multivalent displays of the glycomimetics were found to engage DC-SIGN and elicit signaling and receptor-mediated internalization demonstrating the glycomimetics are capable of acting as agonists of DC-SIGN. These findings demonstrate that compounds derived from (-)-shikimic acid are worthy of the name “glycomimetic.” Moreover, we have generalized this strategy by developing a divergent synthesis of shikimic acid epimers that serve as scaffolds to mimic L-fucose, D-galactose, and D-glucose. These selective glycomimetic agonists will be invaluable for elucidating the role a specific CLR plays in immunity.

Development of α/β peptides to mimic glucagon-like peptide 1 (GLP-1)

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Modulating protein-protein interactions (PPIs) is an active goal with both fundamental and therapeutic motivations. α/β peptides have been effectively used to interfere with PPIs involving helical contact areas. Glucagon-like peptide 1 (GLP-1) is a helix-containing peptide hormone that promotes glucose-stimulated insulin secretion, glucose regulation, and proper β -cell function. Binding of GLP-1 to the GLP-1 receptor (GLP1-R), a class B GPCR, recruits several different intracellular proteins, including $G\alpha_s$, β -arrestin 1 and β -arrestin 2, to the GLP-1R. We have developed an α/β peptide mimic of GLP-1 that is able to activate the GLP-1R to $G\alpha_s$ signaling, potentiate insulin secretion and regulate blood glucose as effectively as GLP-1. A bioluminescence resonance energy transfer (BRET) assay has been developed to assess the ability of the above α/β peptide to recruit β -arrestins to the GLP-1R. Using BRET, we have found that this α/β peptide does not activate other signaling pathways activated by the interaction between GLP-1 and GLP-1R, suggesting that the α/β peptide is a highly biased agonist. Recent work toward characterizing the biased nature of this ligand will be discussed.

Development of in vitro polyketide synthase catalysis and evaluation of substrate promiscuity

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Type I polyketide synthases from the pikromycin pathway have been developed in vitro via substrate engineering and leveraged to synthesize 12- and 14-membered ring macrolides.¹ A panel of unnatural substrates has been synthesized for a "torture test" to ascertain the domain dependence of substrate promiscuity, toward rational protein engineering of these complex enzymes.

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Reconstitution and single molecule characterization of yeast spliceosomal commitment complex

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Prior to translation in eukaryotic cells, the intronic regions of precursor messenger RNA (pre-mRNA) must be removed and the protein-encoding exons ligated together to generate mature mRNA. This process is performed by the spliceosome, a macromolecular machine composed of five small nuclear RNAs (snRNAs) and more than 100 proteins in *Saccharomyces cerevisiae*. One of the earliest stages in spliceosome assembly on pre-mRNA is the formation of commitment complex (CC). Genetic and biochemical studies suggest that the nuclear cap binding complex (CBC) and the branch point binding heterodimer Msl5•Mud2 stabilize the otherwise transient association of U1 small nuclear ribonucleoprotein (snRNP) complex to the 5' splice site of pre-mRNA to form commitment complex. However the intrinsic pre-mRNA-binding ability of U1 snRNP and the contributions of CC components to the stability of this interaction have not been quantified, in part because CC has never been reconstituted separate from other spliceosomal components. Here we present work towards the characterization of commitment complex by Colocalization Single Molecule Spectroscopy (CoSMoS) experiments with purified and fluorescently labeled U1 snRNP. We isolated U1 snRNP from all other spliceosomal proteins by a novel purification method and labeled the complex with a tri-functional fluorescent SNAP ligand for single molecule study. Results from biochemical and mass spectrometry experiments show that the complex is purified, intact, and functional for splicing. Preliminary CoSMoS data suggest that purified U1 snRNP can associate with fluorescently labeled pre-mRNA constructs and is competent for single molecule experiments which elucidate the kinetics of early spliceosome assembly.

Towards the Design of Effective Antibacterial Agents

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Pathogenic infections represent a persistent threat to human health. The rapid development of resistance to drug therapies creates a continuing need for developing new anti-infective agents. Host-Defense Peptides (HDPs) represent a potential source of inspiration for development of new antibacterial agents. Elucidation of high-resolution structure has been challenging for the large group of HDPs. We used racemic crystallization to obtain the crystal structure of an analogue of the widely-studied HDP, magainin 2. The crystal structure features a dimerization mode that has previously been proposed to play a role in the antibacterial activity of magainin 2 and related peptides (1).

The broad molecular diversity among HDPs suggests that their prokaryotic-selective activity is not tightly coupled to specific features of amino acid sequence or peptide conformation. This situation has inspired the development of several families of sequence-random hydrophobic-cationic co-polymers that display antibacterial behavior with varying levels of hemolytic activity. We employed solid-phase synthesis in an unconventional way to generate peptide mixtures that contain one type of hydrophobic residue and one type of cationic residue. Each mixture was random in terms of sequence, but highly controlled in terms of chain length and stereochemistry. Analysis of the antibacterial and hemolytic properties of these mixtures revealed that selective antibacterial activity can be achieved with heterochiral binary mixtures but not homochiral binary mixtures (2).

We have explored nylon-3 materials (poly- β -peptides) as functional mimics of HDPs (3, 4). Nylon-3 polymers are readily synthesized via ring-opening polymerization of β -lactams; some hydrophobic-cationic copolymers display broad antimicrobial activity. It is challenging to perform structure-activity relationships studies for nylon-3 copolymers (or other copolymers) because each sample contains many different kinds of molecules (different lengths, different subunit compositions, different subunit sequences, different subunits stereochemistries). Using our unconventional solid-phase approach we were able to prepare nylon-3 copolymer mixtures that are better defined than are the mixtures generated via ring-opening polymerization of β -lactams. This synthetic strategy is not available for any other types of polymers that have been reported to function as selective antibacterial agents. Our findings show that chain length and stereochemistry are important parameters in terms of determining the activity of polymers of this type.

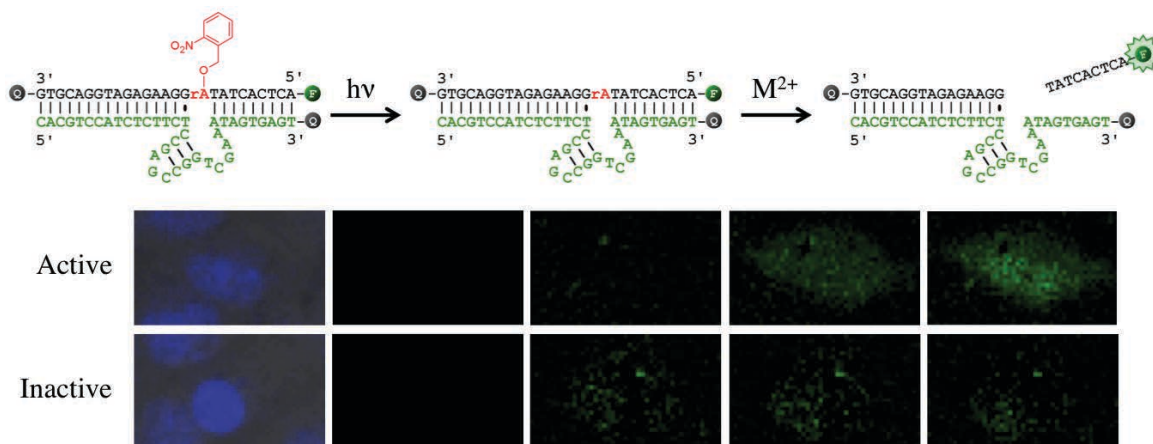
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Caged DNAzymes for Cellular Metal Ion Sensing

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Metal ions are critical for biological function and their levels are tightly controlled. The development of sensors capable of probing cellular levels of metal ions has long been an active area of research. Recently DNAzymes, sequences of DNA with catalytic activity, have been demonstrated as a potential platform for metal ion detection, bearing advantages in terms of selectable metal ion specificity, ease of synthesis and modification, and water solubility. However, despite this promise, cellular sensing of biologically prevalent metal ions using DNAzymes has not yet been possible, in part due to the ‘always-on’ nature of first generation DNAzyme sensors. In the current work we demonstrate the design and synthesis of a photoactivatable (‘caged’) DNAzyme, which we apply towards the sensing of Zn(II) within live cells. The incorporation of a photolabile group allows for the inhibition of DNAzyme activity in the presence of its target as well as protection from degradation within cell lysate. Irradiation with UV light is able to remove this functional group, restoring DNAzyme activity and allowing for control over the sensing ability of the sensor. We are able to show that this strategy applies to a range of DNAzymes with different metal ion cofactor requirements, demonstrating the generalizability of the strategy and allowing the detection of many different metal ions within cells.



O-GlcNAc Transferase Makes the Cut

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Abstract: O-GlcNAc Transferase (OGT) is an essential human enzyme that catalyzes the addition of a single *N*-acetylglucosamine to serine or threonine residues of intracellular proteins. This unique post-translational modification modulates a wide range of signaling pathways linked to glucose metabolism and its abnormality has been linked to many human diseases. Recently, OGT has also been suggested to participate in the proteolytic maturation of Host Cell Factor 1 (HCF-1) – an important regulator of the cell cycle. However, the role of OGT in the proteolysis of HCF-1 remains largely unknown. This poster will present the elucidation of this unique activity of OGT by using biochemistry, mass spectrometry and X-ray crystallography. This study solves a long-standing biological mystery, while expanding our knowledge of the already impressive regulatory mechanisms of OGT in different life processes.

An Untargeted Metabolomics Approach for Discovery of Novel Natural Products from Genetically-Perturbed *Streptomyces*

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Natural products represent a significant proportion of current therapeutic agents and although it has been demonstrated that the biosynthetic potential of bacterial sources remains largely untapped, discovery efforts have slowed in recent decades. This is due in large part to the difficulty and cost of traditional isolation strategies that depend upon bioactivity-guided purification before compound characterization can be performed. This strategy often leads to the rediscovery of previously-characterized compounds, as well as the omission of low-abundant compounds and those not active in the chosen bioactivity screen. We have developed a mass spectrometry-based, untargeted metabolomics approach that facilitates characterization of secondary metabolites directly from crude extracts with no prior purification. We rapidly exclude known metabolites using accurate mass databases and focus MSⁿ fragmentation characterization efforts on potentially novel molecules.

We have applied our analysis approach to extracts of *Streptomyces coelicolor* M145 with perturbations to *crp*, a global regulator of secondary metabolism. Comparative mass spectrometry software allows the prioritization of compounds based on their likely novelty (*i.e.*, a compound produced in a perturbed strain but not in the wild type). Previously characterized compounds are excluded through database searching and remaining leads characterized with a variety of mass spectrometry-based techniques, including MSⁿ fragmentation and ion mobility spectrometry. Importantly, our analyses are performed directly on crude bacterial extracts meaning that known metabolites are excluded early in the procedure, reducing the probability of compound rediscovery. We have thus far identified several potentially novel metabolites including hydroxamate siderophores, prodigiosin analogues, and those with no apparent similarity to known compounds. Further characterization of these molecules and their functional roles in *S. coelicolor* is currently underway.

Chemical Biology Probes for The SUMO protease SENP1, a Novel Target for Prostate Cancer Therapy

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Androgen signaling through the androgen receptor (AR) is essential for normal growth and function of the prostate gland. In prostate cancer (PC), androgens provide the main proliferative drive for the disease, making androgen-deprivation one of the primary therapies. Although initially effective, such treatments select for tumor cells that are able to sustain proliferation in a reduced androgen environment. This allows for the emergence of castration resistant PC, an incurable disease where both the AR transcriptional program is subverted and cellular senescence is evaded. SUMOylation is a post-translational modification that regulates both of these processes. SUMOylation of AR inhibits both basal and androgen-stimulated transcription in a promoter context manner and enhanced global SUMO modification induces prostate cell senescence. Advanced PC cells evade these mechanisms through the upregulation of SENP1, a SUMO-specific cysteine protease that reverses SUMOylation. Since AR is a direct activator of the SENP1 gene, this creates a self-reinforcing loop that promotes PC progression. Our objective is to reverse this process through the discovery and development of small molecule inhibitors of SENP1. Using a robust FRET-based assay, a high throughput screening campaign led to the identification of multiple SENP1 inhibitors that act in both a reversible and competitive manner. These hits have been further characterized for selectivity towards other SUMO and Ubiquitin proteases. Several scaffolds show activity towards natural substrates in complex protein mixtures and are amenable to further development. We are examining the basis for selectivity and characterizing the activity of lead inhibitors towards prostate cancer cells to evaluate the therapeutic potential of this novel approach alone or in combination with existing endocrine therapies.

Using GlfT2 point mutants to interrogate galactan chain length in *Mycobacterium smegmatis*

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is a disease that has afflicted humanity for over 17,000 years, but only in the last 70 years has it become curable. The advent of MDR (multi-drug-resistant) TB has necessitated more research into developing novel tuberculosis therapeutics. In the past, one strategy that has paid dividends has been to target the enzymes necessary for the construction of the complex, mycobacterial cell wall. Better understanding this cell wall could provide valuable insight into devising new treatments for tuberculosis. One of the integral components of this cell wall is the galactan, a polymer of repeating galactofuranose monosaccharides. Though the usual length of the galactan is between 35-40 residues, the only known function of this polymer is to create three arabinofuranose branch points within the first 12 galactofuranose residues. Our goal is to better understand how altering galactan chain length affects mycobacteria *in vivo*. GlfT2 is the mycobacterial galactofuranosyl transferase responsible for the construction of this chain. *In vitro* studies have revealed point mutants which result in shorter polymer products. We propose these point mutants have reduced affinity for the UDP-galactofuranose donor, ultimately resulting in shorter chain length. Through genetic recombination and CRISPR-mediated genome editing, we seek to investigate galactan chain length in *M. smegmatis*.

Covalent inhibitors of HECT E3 Nedd4-1 processivity discovered with irreversible tethering

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HECT E3 ubiquitin ligases are implicated in a variety of human diseases, but there are no reported small molecule inhibitors of these enzymes. In order to discover new inhibitors of the HECT E3 Nedd4-1, which has a catalytic and non-catalytic surface cysteine, we developed a novel irreversible fragment tethering approach. This method employs the irreversible covalent trapping of drug-like fragments at surface cysteines. We rationally designed a chemical system to attach a cysteine-reactive electrophile to 100 drug-like fragments without significant alterations in the thiol reactivity of the attached electrophile, ensuring that specific binding and not increased reactivity will produce candidate inhibitors. We employed a simple and high throughput mass spectrometry based assay to screen our library in mixtures of ten fragments and discovered two fragments which reacted with Nedd4-1. These fragments were selective for Nedd4-1, and did not cross react with other E2s, E3s, deubiquitinases, and cysteine proteases. Surprisingly, we found that these inhibitors did not react with the more reactive catalytic cysteine of Nedd4-1, but another surface cysteine near the non-covalent ubiquitin binding site. However, due to their proximity to the non-covalent ubiquitin binding site, these covalent inhibitors reduce the binding affinity of Nedd4-1 for ubiquitin, which is essential for polyubiquitination but not monoubiquitination. *In vitro* enzymatic assays have shown that these molecules inhibit Nedd4-1 polyubiquitination processivity. The X-ray crystal structure of the most potent inhibitor in complex with Nedd4-1 has been solved, demonstrating that it forms a stable covalent bond with the ubiquitin-binding site cysteine of Nedd4-1 and has specific interactions with residues around this cysteine. This structure will be used to further optimize the fragment into a more potent inhibitor, which can then be used to study Nedd4-1 function in cells. In particular, it will be used to help resolve the controversy over whether Nedd4-1 polyubiquitinates the tumor suppressor PTEN. It can also be optimized to be used as a therapeutic for the many cancers and neurodegenerative diseases in which Nedd4-1 polyubiquitination is implicated, without disrupting the essential cellular processes dependent on Nedd4-1 monoubiquitination.

Virtual screening for competitive UDP-galactopyranose mutase ligands identifies novel class of antimycobacterial agents

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Uridine 5'-diphosphate (UDP) galactopyranose mutase (UGM) catalyzes the formation of UDP-galactofuranose, the nucleotide activated precursor for incorporation of galactofuranose into cell surface glycoconjugates of several pathogens. UGM is essential for viability of the human pathogen *M. tuberculosis*, making it an attractive therapeutic target for treatment of tuberculosis. Previous attempts to identify UGM inhibitors using high throughput screening (HTS) have met limited success. As an alternative to the confined chemical libraries and stringency of HTS, we turned to virtual screening. Small molecule UGM ligands were predicted *in silico* and top-ranking molecules were tested empirically. Utilizing this approach, we have identified a novel series of competitive UGM inhibitors that display potent inhibition of UGM *in vitro* and exude antimycobacterial activity. In addition, we have determined the first UGM-small molecule co-crystal structure, guiding further optimization of this inhibitor series. These inhibitors may serve as tools to probe the role of UGM in biological systems, as well as act as therapeutic leads for treatment of mycobacterial diseases.

New Roles for Oxygen-Activating Diiron Enzymes in Antibiotic Biosynthesis

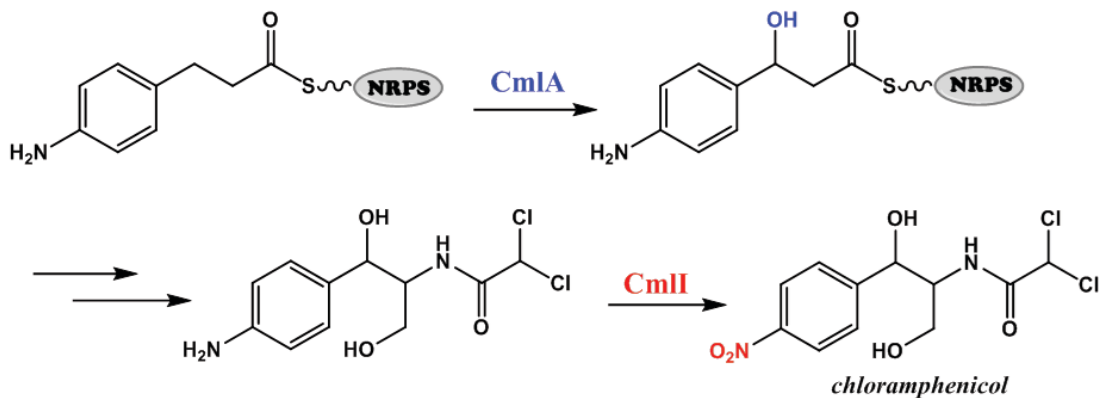
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Abstract: The emerging roles of oxygen-activating diiron enzymes in antibiotic biosynthesis are exemplified by two enzymes involved in the biosynthesis of the broad-spectrum antibiotic chloramphenicol: the β -hydroxylase CmlA that works in concert with the nonribosomal peptide synthetase (NRPS) CmlP,¹ and the NH_2 -oxygenase CmlI that catalyzes the conversion of an arylamine precursor to the final aryl nitro group-containing chloramphenicol.² The diiron active site of CmlA is bound in a metallo- β -lactamase protein fold unusual to diiron enzymes and ligated by three histidine and three carboxylate residues, as revealed by a recent 2.17 Å resolution crystal structure.³ Although no crystal structure is available for CmlI, comparison to an enzyme with a homologous sequence suggests a three-histidine four-carboxylate protein architecture.⁴ These ligation strategies are in contrast to the two-histidine four-carboxylate protein architecture common to canonical diiron enzymes such as methane monooxygenase and ribonucleotide reductase. In addition to structural novelty, both CmlA and CmlI have unusual reactivities. CmlA is the first known diiron catalyst involved in amino acid β -hydroxylation on NRPS biosynthetic pathways, a reaction typically associated with α -ketoglutarate-dependent nonheme monoiron enzymes or cytochrome P450 enzymes. CmlI is one of only three known enzymes that performs aromatic n-oxygenation, and thus is of interest to both biochemists and synthetic chemists. Kinetic and spectroscopic studies that address both structure and mechanism of these enzymes will be discussed.

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Structural Characterization of Ubiquitin Trimers using Small Angle X-Ray Scattering and Molecular Dynamics Simulations

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The etiology of many human diseases can be traced to aberrant ubiquitin (Ub) posttranslational modifications (PTMs). A large network of Ub ligases, deubiquitinases (DUBs), and proteins with Ub receptors govern nearly every biological pathway in eukaryotes. At the heart of these networks lies a vast array of Ub PTMs ranging from monoUb to oligomeric Ub chains of varying topology. Indeed, we are just beginning to appreciate that topologically distinct Ub chains provide spatial and temporal control over different biological pathways. Yet we are far from understanding how various DUBs and Ub receptors interpret the information stored in different Ub modifications. In this regard, we aim to shed light on the structural aspects of ubiquitin chains through molecular dynamics (MD) and small angle X-ray scattering (SAXS) methods. The general approach is to integrate MD simulations at atomistic level to sample the conformational ensemble, which is then filtered/refined with experimental SAXS data. This general strategy has proven successful in several previous studies of multi-domain proteins [1-4]. These studies relied purely on coarse-grained (CG) models to generate the conformational ensemble. Our approach will also take advantage of recent advances in molecular dynamics simulations using GPUs [5], which routinely allow atomistic, implicit solvent simulations at the microsecond time scale. In the long run, implementation CG models for long Ub chains will allow us further computational efficiency. A critical comparison of the atomistic and CG ensembles in the context of low-resolution data such as SAXS will further enhance confidence in the computational results.

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**A Small Molecule That Displays Marked Reactivity Toward Copper-
versus Zinc Amyloid- β Implicated in Alzheimer's Disease**

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Alzheimer's disease (AD) is a complex, multifactorial, neurodegenerative disease that poses tremendous difficulties in pinpointing its precise etiology. A variety of factors, including protein aggregates (both of A β and hyperphosphorylated tau) and dysregulated metal ions, have been suggested to contribute to the disease's onset and progression. Further exacerbating the complexity of the disease, it has been demonstrated that metal ions (especially Cu(I/II) and Zn(II)) are able to interact with A β and alter its aggregation. A toolkit that specifically targets and modulates individual key players in AD may elucidate their roles in disease onset and progression. We report high-resolution insights on the activity of a small molecule (**L2-NO**) which exhibits preferential reactivity toward Cu(II)-amyloid- β (A β) over Zn(II)-A β and metal-free A β .

A New Binding Site on E6AP for UbcH7

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In cervical cancer, the E3 ligase E6AP ubiquitinates the tumor suppressor p53. Before this happens, the E2 UbcH7 loads E6AP with ubiquitin (Ub). Although a co-crystal structure indicates a single binding site on E6AP for UbcH7,¹ recent kinetic work hinted that E6AP encounters UbcH7 at two distinct binding sites.² In developing custom photo-cross-linkers, we confirmed the secondary site and are identifying its residues with mass spectrometry. We mono-alkylated UbcH7 cysteine point mutants with an iodoacetamide diazirine, and then cross-linked them to E6AP under UV irradiation. Since UbcH7 F63A has much reduced affinity for E6AP (800 μ M rather than 5 μ M), we used it to test for cross-linking specificity. While neither UbcH7 F63A, nor UbcH7 F63A-Ub (UbcH7 charged with Ub through oxyester) cross-linked E6AP (Fig. 1A, lanes 5-7), UbcH7 F63A-Ub cross-linked E6AP in the presence of non-cross-linkable wild type UbcH7-Ub (Fig. 1A, lane 8). Thus, we propose that E6AP binds UbcH7~Ub at the canonical site before binding a second equivalent of UbcH7~Ub at the noncanonical site (Fig 1E). We will present tandem MS identification of cross-linked residues from the canonical binding site on E6AP (Fig. B and C), and from the noncanonical site (Fig. D). Overall, this work has discovered a previously unknown allosteric binding site on E6AP that opens when the canonical site is occupied by Ub-charged UbcH7.

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A New Binding Site on E6AP for UbchH7

David T. Krist*, Alexander V. Statsyuk
continued

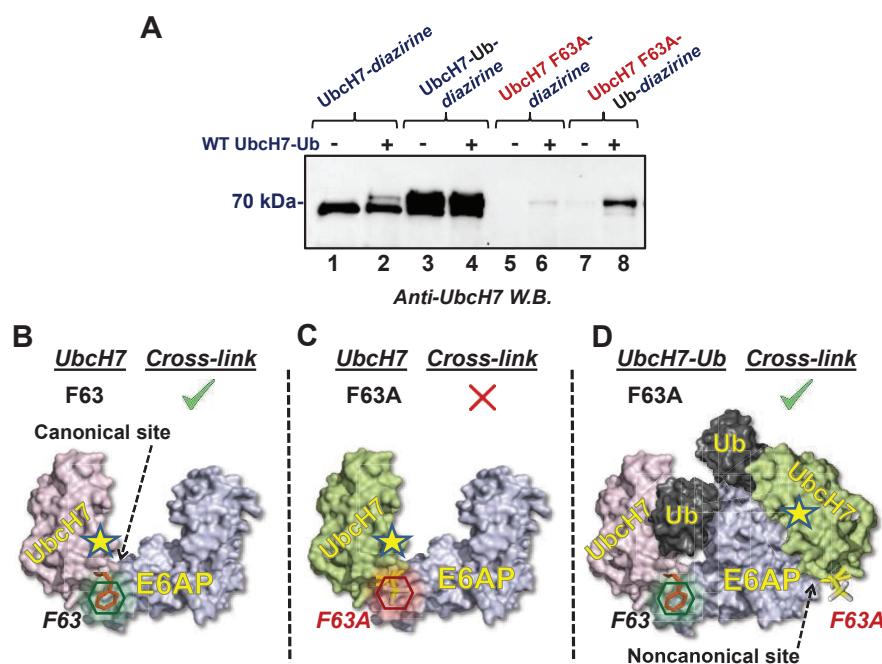


Figure 1. Detection of an allosteric binding site on E6AP for UbchH7 (A) Western blot bands indicate a successful cross-link between E6AP and UbchH7 site-specifically modified by a diazirine-bearing photo-cross-linker. (B-D) Proposed protein orientation during cross-linking (PDB: 1C4Z). UbchH7 is mono-alkylated with the diazirine-bearing cross-linker at a residue indicated by the star. UbchH7 is docked at the canonical site indicated by the original co-crystal structure in B and C. In D, E6AP undergoes a conformational change to open a noncanonical binding site.

Functional Reverse Translation Via DNA-encoded Proteomic Probes

Rachael R. Jetson and Casey J. Krusemark

Because DNA is the central information storage molecule of biology, powerful tools exist to read, write, and manipulate DNA-encoded information. These include both enzymes from nature (DNA polymerase, restriction enzymes) and man-made tools (DNA synthesis chemistry, DNA sequencers). The power of these tools is well demonstrated in the cloning, sequencing, and characterization of RNA made possible by the reverse transcription of RNA to DNA sequence.

We present DNA-encoded probes of proteome function to enable the encoding of proteomic information in DNA sequence, thereby accomplishing a functional reverse translation. Probes are composed of an enzyme substrate linked to an encoding DNA scaffold. Modification of the substrate to product allows the selective addition of an affinity tag for physical separation of substrate and product probes. The amount of DNA in the purified product fraction correlates to the enzyme activity of a sample. We demonstrate the design and application of probes for detection of several enzymatic activities (kinase, protease, and farnesyltransferase activities) using quantitative PCR.

Further developments include the design of large suites of probes to measure numerous enzymatic activities and protein abundances and storage of this information in a functional proteomic DNA (fpDNA) library. In addition to proteome characterization, we demonstrate utility of the approach towards discovery of active molecules in high-throughput screening (HTS) through detection of perturbations to protein function. The technique will allow many of the benefits realized in the selection or *in vitro* evolution of DNA-encoded, DNA-linked small molecule libraries to be applied to conventionally synthesized, commercially available HTS collections.

Covalent Small Molecule Modulators of Coactivator-Activator Interactions

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Due to the importance of protein-protein interactions (PPIs) in biological processes they are attractive targets for small molecules to use as mechanistic and therapeutic tools. However PPIs with transient, low affinity contacts over large, shallow surfaces are often intractable targets for small molecules. Examples of these difficult PPIs are the complexes formed between activators and coactivators. Specificity also hampers the development of small molecules for use as mechanistic probes. For instance the KIX domain of the coactivator CBP (CREB-binding protein) is also found in its homolog p300 and in the Mediator subunit MED15. Here we apply the Tethering strategy to match orthogonal small molecule modulators with target sites on the coactivator, using the KIX domain of CBP as a model. The KIX domain uses two distinct binding surfaces connected by an allosteric network to interact with more than a dozen transcriptional activators including MLL and c-Jun at one site and c-Myb or CREB at the second. Building upon a previous liquid chromatography mass spectrometry-based Tethering screen to target the MLL-binding site of the KIX domain, a novel FP-based screen streamlined the identification of small molecule inhibitors directed at the c-Myb and CREB-binding site of the KIX domain. A disulfide-containing molecule was converted into a series of covalent irreversible probes which bound specifically to the target site within the KIX domain. These orthosteric inhibitors also functioned as allosteric modulators providing additional control over the interactions formed between the KIX domain and its activator binding partners. Tethering will be extended to assess the role of CREB and CBP and its homolog p300 in cells.

A Potent, Versatile Disulfide-Reducing Agent from Aspartic Acid

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Dithiothreitol (DTT) is the standard reagent used for the reduction of disulfide bonds in biological molecules. But with thiol pK_a values of 9.2 and 10.1, the molecule is mostly inactive at neutral pH. Herein, we report on (2*S*)-2-amino-1,4-dimercaptobutane (dithiobutylamine or DTBA), a dithiol that is easily accessed from L-aspartic acid. DTBA possesses thiol pK_a values that are approximately 1 unit lower than those of DTT and forms a similar six-membered dithiane ring upon oxidation. DTBA reduces disulfide bonds in both small molecules and proteins faster than does DTT. We also note that the amino group on DTBA facilitates its covalent attachment to a resin, surface, or soluble molecule. These attributes of DTBA could enable it to supplant DTT as the preferred reagent for reducing disulfide bonds in biomolecules.

Nitrodibenzofuran: an Efficient One- and Two-Photon Sensitive Photoremovable Protecting Group for Thiol Protection in Peptides

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Photoremovable protecting groups are important for a wide range of applications in peptide chemistry. For thiol protection, the most widely used approach involves masking with an o-nitrobenzyl group. In earlier work, we demonstrated that the brominated hydroxycoumarin (Bhc) group could be used for thiol group protection in peptidomimetic enzyme inhibitors. The two-photon excitation properties allowed that group to be efficiently removed for biological applications in cell culture. However, interestingly, the use of the Bhc group in peptides is complicated by the formation of an isomeric product that occurs in the photolysis reaction. We have recently characterized that compound. To circumvent that problem, we have prepared cysteine-containing peptides where the thiol is protected with a nitrodibenzofuran (Ndbf) group. That was accomplished by preparing Fmoc-Cys(Ndbf)-OH and incorporating the residue into peptides by standard solid phase peptide synthesis. Irradiation with 360 nm light gave clean conversion to the free thiol. Deprotection was also obtained via two-photon excitation with 800 nm light. Thiol group uncaging was carried out on peptides that are substrates for protein farnesyltransferase in the presence of the enzyme resulting in the formation of prenylated products. Those experiments indicate that deprotection can be performed under mild conditions that allow enzymatic activity to be retained suggesting that the Ndbf group should be useful for caging thiol groups in peptides used for biological experiments.

Human Hints: Powerful Tools for Nucleotide Monophosphate Drug Activation

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The three human nucleotide binding proteins (hHints) are a family of histidine triad proteins which, while their functions have not yet been completely defined, are unique due to their phosphoramidase activity. This inherent activity is enigmatic because phosphoramides are not a common class of molecules produced endogenously in the human body; however, it provides an exciting target for the development of nucleotide monophosphate prodrugs as phosphoramidate monoesters. With the FDA approval of sofosbuvir, a new nucleotide monophosphate phosphoramidate for the treatment of chronic Hepatitis C virus infection—activated to corresponding nucleotide via a hHint1-catalyzed hydrolysis—there is an impetus to investigate the capacity of hHint enzymes to activate other phosphoramidate nucleotides of therapeutic value.

A series of structural studies with commercially available anti-cancer and antiviral nucleosides are described that help to define the structural basis of substrate selectivity by hHints. High resolution crystallographic complexes with both hHint1 and hHint2 enzyme forms are compared, and the influence on binding geometry of non-standard nucleobases and ribose analogs featured in many therapeutic nucleotides is presented. It is anticipated that studies of substrate selectivity can be coupled with knowledge of tissue-specific abundance of hHint enzymes to create a strategy for both broad intracellular de-protection and tissue-selective activation of nucleotide monophosphate therapeutics.

Elucidating the Roles of Glycosaminoglycans in Human Pluripotent Stem Cell Self-renewal and Differentiation

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The development of chemically defined environments for the culture and differentiation of human pluripotent stem cells (hPS) is critical to exploit their full potential for regenerative medicine. The focus has been on media that promote self-renewal or differentiation, but the insoluble substrata also have an important role. In seeking surfaces for hPS cell expansion, the Kiessling group found that integrin-binding surfaces were not adequate but chemically defined surfaces displaying a glycosaminoglycan (GAG)-binding peptide from vitronectin are highly effective for long-term hPS cell culture. These findings implicate GAGs in hPS cell self-renewal. GAGs are anionic polysaccharides that mediate cell-cell and cell-ECM interactions, whose role in hPS cells is poorly understood.

To gain structural insight of the GAGs with which the synthetic surfaces interact, we screened a library of GAG-binding peptides for their ability to adhere hPS cells. From this screen we identified the GAG-binding peptide, RKGSGRRLVK, derived from thrombospondin (TSP). A carbohydrate array displaying defined heparin oligosaccharides found that GKKQRFRRNRKG and RKGSGRRLVK have affinity toward IdoA2S-containing oligosaccharides, suggesting that these peptides target similar GAGs. GAG isolation and structural analyses of H7, H9, H14, and IMR90-4 lines are currently being performed to gain additional structural information. Further studies using GAG knockout lines will determine the functional roles of GAGs and how they may influence cell fate decisions.

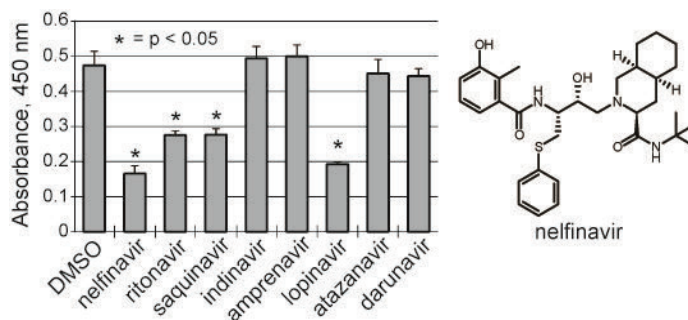
HIV Protease Inhibitors as Probes of Virulence Factor Production and Function

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Bacterial virulence factors are often critical in a pathogen's ability to successfully infect a host. However, the exact role many virulence factors play in pathogenesis remains poorly understood. Chemical inhibitors of virulence factor production or action offer a means to gain insight into the function of virulence factors in addition to serving as potential alternative antibiotics targeting virulence rather than essential life functions. We have identified the HIV protease inhibitor nelfinavir as an inhibitor of the production of streptolysin S (SLS), a posttranslationally modified ribosomal peptide cytolysin produced by *S. pyogenes*. Initial work has indicated that the target of inhibition is a membrane-bound protease. To elucidate the specific target, a medicinal chemistry effort was undertaken to determine structure-activity relationships (SAR) for nelfinavir and resulted in compounds with 10-fold increased potency. Knowledge gleaned from SAR data has facilitated the synthesis of a photoactivatable-cross-linking derivative of nelfinavir, which is being used to identify the target of inhibition by affinity purification.

SLS is a member of a large family of natural product peptides containing thiazole and oxazole rings. Nelfinavir was found to also inhibit the production of the family member listeriolysin S, a closely related toxin from *L. monocytogenes*. To investigate whether nelfinavir could inhibit distantly related family members, it was tested against and found to inhibit the production of the antibiotic plantazolicin as well. These results indicate that nelfinavir has potential as a chemical knockdown probe for natural product discovery as well as serving as a tool to further explore the biosynthesis of these products.



Competition between arachidonic acid and endocannabinoids for oxygenation by cyclooxygenase-2

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Cyclooxygenase-2 (COX-2) catalyzes the *bis*-dioxygenation of arachidonic acid (AA) to PGG₂ and its reduction to PGH₂, the committed steps in prostaglandin (PG) biosynthesis. COX-2 also oxygenates the endocannabinoids, 2-arachidonoylglycerol (2-AG) and arachidonylethanolamide (AEA), leading to the formation of prostaglandin glycerols (PG-Gs) and prostaglandin ethanolamides (PG-EAs), respectively. Purified COX-2 utilizes AA and 2-AG with approximately equivalent efficiencies as judged by k_{cat}/K_m values for oxygenation. However, the cellular levels of PG-Gs are 2-3 orders of magnitude lower than PGs. The cause of this dramatic difference in PG-G levels produced by pure COX-2 versus cellular COX-2 has remained uncertain.

Kinetic studies with purified mCOX-2 revealed that AA is a potent, non-competitive inhibitor of 2-AG and AEA oxygenation, as revealed by reductions in V_{max} but not in K_m . 2-AG and AEA are weak inhibitors of AA oxygenation by mCOX-2. The effect of AA levels on endocannabinoid oxygenation in intact cells was evaluated in RAW264.7 macrophage and 3T3 fibroblast cell lines. Reduction in AA levels, resulting from treatment with the cPLA₂α inhibitor, giripladib, correlated with a >10-fold increase in 2-AG oxygenation as judged by PG-G production. Antagonism between AA and 2-AG for oxygenation by COX-2 may account for the higher levels of PGs compared to PG-Gs in cellular settings. These findings enable a more thorough understanding of the disparities in endocannabinoid- and arachidonic acid-derived prostaglandin levels and demonstrate conditions whereby endocannabinoid oxygenation can be enhanced.

Supported by research and training grants from the NIH (GM15431 and T32 GM065086).

Diazo Compounds for Bioversible Esterification of Proteins

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Chemoselective reactions now enable the modification of natural amino-acid side chains as well as those bearing a non-natural moiety, such as an azido group. These reactions typically lead to the irreversible formation of a product. Although this chemical stability is often desirable, a method that produces a “bioreversible” linkage would be useful for many applications. This concept mimics the action of prodrugs, in which cellular enzymes unmask an inactive precursor to reveal an active small-molecule drug.¹ We hypothesize that this concept can be applied to proteins by esterifying carboxylic acids with diazo compounds. Although diazo compounds have found widespread utility in synthetic organic chemistry, their application in chemical biology has been sparse. Attempts to label proteins using diazo compounds has been inefficient, even in the presence of a vast excess of diazo compound.² We have shown that this problem can be overcome by tuning the pK_a of the parent compound such that the diazo compound is reactive enough to esterify the acid while maintaining selectivity for esterification over hydrolysis.³ An optimal diazo compound is being identified, and that compound will be decorated with moieties of use in basic research as well as protein-based therapeutics. This work is being supported by grant R01 GM044783 (NIH).

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Active Efflux Influences the Activity of Quorum Sensing Inhibitors in *Pseudomonas aeruginosa*

Joseph D. Moore, Joseph P. Gerdt, Nora R. Eibergen, and Helen E. Blackwell

Abstract

Many bacteria regulate virulence gene expression through a cell-cell signaling process known as quorum sensing (QS). In *Pseudomonas aeruginosa*, QS is largely mediated by signaling molecules known as *N*-acylated homoserine lactones (AHLs) and their associated intracellular LuxR-type receptors. *P. aeruginosa* controls an arsenal of virulence factors and biofilm formation through QS. The active efflux pump MexAB-OprM has been previously shown to secrete a native *P. aeruginosa* signaling molecule, *N*-3-oxo-dodecanoyl-L-homoserine lactone, and in the current study we show that MexAB-OprM additionally recognizes a variety of AHL-based QS inhibitors. We also demonstrate that the potency of 5,6 dimethyl-2-aminobenzimidazole as a QS inhibitor is not affected by the presence or absence of the MexAB-OprM pump. These results have implications for the use of non-native AHLs and related derivatives as QS modulators and provide a potential design strategy for the development of new QS modulators that are resistant to active efflux.

RNA polymerase II C-terminal domain phosphorylation mediates the environmental stress response in yeast

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The carboxyl-terminal domain (CTD) of the largest subunit of RNA polymerase II consists of a highly conserved repeating heptapeptide (YSPTSPS). Reversible phosphorylation of these residues controls the recruitment and assembly of machinery involved in transcription regulation. Previous studies have elucidated the roles of Tyr1, Ser2, Ser5, and Ser7 phosphorylation, but the role of Thr4 phosphorylation remains poorly characterized. Here we identify multiple kinases that phosphorylate Thr4 in vitro. Interestingly, many of these kinases are part of a larger signaling network that constitutes the environmental stress response (ESR), a signaling cascade that results in the up- or down-regulation of a subset of genes necessary to protect the cells during stress. Previous studies indicate that these gene expression changes are mediated solely by changes in the activation state of transcription factors. However, here, we show that post-translational modifications to the RNA polymerase II CTD itself mediate the environmental stress response in yeast.

Electronic Effects on Biomolecular Structure

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The unique conformations of biological molecules enable their specific activities and arise from the cooperation of a wide variety of noncovalent interactions, chiefly hydrogen bonding, electrostatic interactions, and van der Waals' forces. Recently, interactions involving electron delocalization have also been posited to contribute to the observed conformations of biomolecules. In particular, donation of electron density from the lone pair (n) of a carbonyl oxygen into the empty π^* antibonding orbital of another, nearby carbonyl group has gained great attention. We show that such $n \rightarrow \pi^*$ interactions between carbonyl groups are distinct from electrostatic or dipolar interactions and that they have energies upwards of 0.27 kcal/mol. We also show that these interactions can be enhanced by substituting the carbonyl oxygen for sulfur and demonstrate how this strategy can be used for evaluating the role of $n \rightarrow \pi^*$ interactions in the structure and stability of proteins. Bioinformatics analysis further demonstrates that these weak but abundant interactions impart distinctive signatures onto the structures and electronics of proteins, highlighting their previously unappreciated influence. Moreover, the impact of these interactions on the structures of organic polymers suggests that these interactions can guide the folding of proteins prior to the formation of native hydrogen bonding patterns. Finally, we demonstrate that an $n \rightarrow \pi^*$ interaction likely affects the potency of bioactive small molecules, specifically the *N*-acyl homoserine lactone mediators of bacterial quorum sensing.

Protein Ubiquitination by HECT E3s without ATP, E1 and E2 Enzymes

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Protein ubiquitination is a highly conserved post-translational modification that regulates a broad range of cellular processes.¹ Conjugation of the ubiquitin to protein substrates requires ATP and a sequential action of three enzymes: ubiquitin activating enzymes (E1s, 2 known), ubiquitin conjugating enzymes (E2s, ~37 known), and ubiquitin ligases (E3s, ~600 known) that select protein substrates and catalyze the isopeptide bond formation.² Such biochemical complexity of the ubiquitin conjugation cascade makes it difficult to study the catalytic mechanism and to discover pharmacological modulators of E3s, since E3s are positioned at the very end of the E1-E2-E3 enzymatic cascade. It is even more challenging to study the multi-subunit E3s such as APC/C E3 and cullin-RING E3s, where functional E3 complexes consist of up to 3-15 protein subunits.³ Therefore, we envisioned that alleviating the complexity of the E1-E2-E3 cascade would create new opportunities to understand the physiological roles of E3s. In this endeavor, we recently developed a two-component ubiquitination system for a model HECT E3 ubiquitin ligase Rsp5.⁴ As opposed to the native system where ubiquitination requires E1, E2, Rsp5, ubiquitin and ATP (5 components), the developed two-component system requires only chemically activated ubiquitin and Rsp5 (2 components). The C-terminus of ubiquitin is activated as a thioester form that can undergo transthioylation reaction with the catalytic cysteine of Rsp5 to directly form Rsp5~Ub thioester catalytic intermediate. The resulting Rsp5~Ub recapitulates native enzymatic reaction that includes autoubiquitination, substrate ubiquitination, and K63-specific polyubiquitin chain synthesis in a mechanism-dependent manner. Here, we present how this novel concept could facilitate studying biochemical mechanism and developing small molecular inhibitors for HECT E3s.

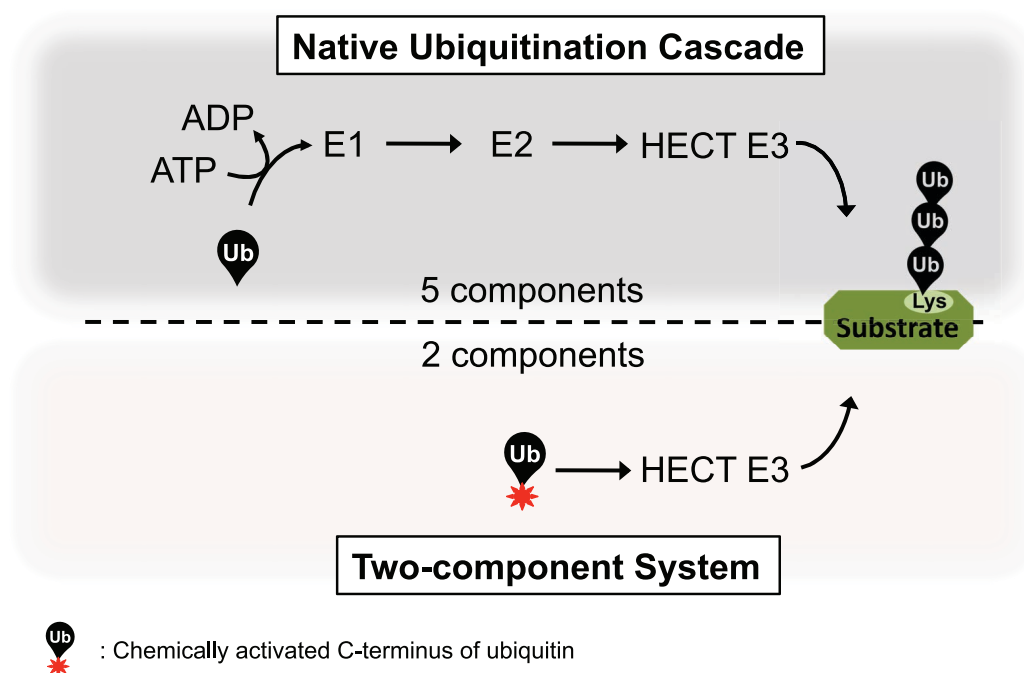
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Protein Ubiquitination by HECT E3s without ATP, E1 and E2 Enzymes

Sungjin Park*, Galyah Boneh and Alexander V. Statsyuk

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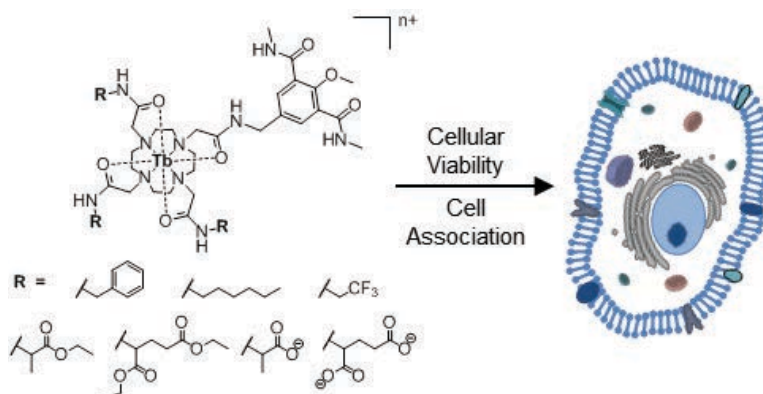


Effect of Lanthanide Complex Structure on Cell Viability and Association

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A systematic study of lanthanide metal complexes that investigates the effect of hydrophobicity and charge on cell viability and cell association is presented. These terbium luminescent probes utilize the macrocyclic polyaminocarboxylate ligand (DOTA) that allows the hydrophobicity of the antenna or that of the carboxamide pendant arms to be varied independently. The *in vitro* function of three sensitizing antenna was assessed: 2-methoxyisophthalamide (IAM(OMe)), 2-hydroxyisothalamide (IAM), and 6-methylphenanthridine (Phen). The highest quantum yield was exhibited by the Tb-DOTA-IAM, but the related Tb-DOTA-IAM(OMe) platform was more attractive for additional studies due to higher cell viability and more facile synthesis. Next, the macrocyclic ligand was functionalized with various carboxamide arms featuring hydrophobic benzyl, hexyl, trifluoro groups and hydrophilic, amino acid based substituents. Regardless of the complex lipophilicity or overall charge, a minimal effect on cell viability was observed ($ED_{50} > 300 \mu\text{M}$) for the entire class of compounds. Only the hexyl substituted complex reduced cell viability to 60 % in the presence of 100 μM complex. Cellular association was examined by ICP-MS and fluorescence microscopy. Interestingly, the hydrophobic complexes did not facilitate an increase in cell association in comparison to the hydrophilic amino acid analogs. The study supports that the hydrophilic nature of the 2-methoxyisophthalamide antenna (IAM(OMe)) disfavors the cellular association of these complexes, allowing responsive probes based on this scaffold to be well suited for the detection of extracellular analytes.



Conformational Dynamics of Ubiquitin Signaling

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Macromolecules such as proteins and DNA are dynamic—they adopt flexible conformations that effect selective recognition by interacting partners. For such dynamic systems, the macromolecule should be presented as an ensemble of conformations rather than a single conformation. This type of approach is especially necessary when conformational differences are closely tied to function, such as in ubiquitin (Ub)-mediated cellular signaling. Conventional methods for structural analysis, such as nuclear magnetic resonance spectroscopy (NMR) and X-ray crystallography, have generated important rigid body models of Ub oligomers. However, such models do not report on the dynamic nature and flexibility of the different Ub chain types. To fill this niche, we use small angle X-ray scattering (SAXS) measurements paired with molecular dynamic (MD) simulations to reveal the conformation ensembles of select Ub chains. We apply this strategy in conjunction with biochemical analysis to illuminate the connections between Ub structures and functions in the cell.

Aromatic Amino Acids: Privileged Side-Chains For Protein-Protein Interaction Inhibitor Discovery.

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This poster describes our recent results developing spectroscopic drug screening methods using ¹⁹F NMR to discover organic molecules for modulating gene regulation of histone-binding proteins and co-activators (e.g., creb-binding protein, CBP). These proteins have pleiotropic roles in transcriptional control, cell growth and development, and are involved in numerous protein-protein interactions (PPIs). The estimated 650,000 PPIs are a powerful means to transfer information within human cells, control protein function, and maintain cell homeostasis. Leading experts Wells and Gestwicki highlight modulation of PPIs as a grand challenge for medicinal chemists and chemical biologists. Transcription factors that form transient interactions ($K_d > 200$ nM) over large surface areas are recognized as some of the most difficult. Robust screening methodologies are in high demand for these PPIs. Chemical probes are essential to further our understanding of biological processes. New methods for initial ligand discovery steps can help change our view of the PPI targetability paradigm and are the essential first step towards probe development.

Given that transcription factors represent a major class of potential drug targets, our proposed ¹⁹F NMR approach to study protein-ligand interactions could significantly increase the repertoire of new targets and thereby open up new avenues for drug/probe discovery. Here we lay the framework for our ligand discovery goals using fluorinated transcription factor PPI domains, showing high incorporation (>90%) of all three fluorinated aromatic amino acids into two different proteins. We determine optimal labeling for developing a sensitive and structure-based method for ligand discovery. We characterize the binding footprints of known ligands and demonstrate our approach in a 500 compound small molecule fragment screen. Finally, using circular dichroism, fluorescence polarization, and x-ray crystallography, we demonstrate the non-perturbing nature of fluorine when placed on aromatic amino acids at PPI interfaces.

Capturing the *In Vivo* Binding Partners of Transcriptional Activators Using a Genetically Incorporated Photocrosslinking Amino Acid

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Protein-protein interactions (PPIs) that involve transient and structurally plastic protein partners, such as that between transcriptional activators and coactivator complexes, have been historically challenging to isolate and characterize. The inherent limitations of many of the currently available approaches for studying PPIs have not readily allowed for the observation of transient PPIs in their native environment. To overcome these limitations, the Mapp lab has employed nonsense suppression to genetically incorporate photolabile unnatural amino acids into yeast proteins, thus allowing for the covalent chemical capture of transcriptional activator-coactivator PPIs in *S. cerevisiae*. For example, we show that the viral activator VP16 directly contacts TBP when the photocrosslinking amino acid *p*-benzoyl-L-phenylalanine (pBpa) is incorporated into the transcriptional activation domain of VP16. Moreover, we demonstrate the utility of a tandem formaldehyde and pBpa crosslinking approach in capturing the direct PPIs of DNA-bound proteins in live yeast. Using this technique, coined tandem reversible irreversible crosslinking (TRIC), we capture the direct binding of VP16 to TBP at the Gal1 promoter, supporting a direct mechanism of recruitment by an activator at this inducible promoter. While the interrogation of specific PPIs yielded key valuable information, high-throughput discovery-mode platforms are also essential for the large-scale identification of novel PPIs. In this regard, we combined pBpa crosslinking and Multi-Dimensional Protein Identification Technology (MuDPIT) mass spectrometry to capture and identify the gamut of interactions maintained by the yeast activator Gal4 *in vivo*. This covalent chemical capture and MuDPIT approach led to the identification of several novel binding partners of Gal4, including two components of the Snf1 kinase complex. Furthermore, using our TRIC method, we were able to capture the direct binding of Snf1 and Gal4 at the Gal1 promoter. Snf1, like the executors of other cellular processes, is part of a multi-component complex that utilizes exchangeable subunits to direct its localization within the cell. Our results support a powerful role for activators in recruiting nuclear-localized, structurally dynamic complexes such as the Snf1 complex. We believe this work should be useful in the examination of other PPI networks that function through dynamic, transient interactions that have been historically challenging to study.

Investigation of G-protein activated inward rectifying potassium channel activator lead to the development of specialized photo affinity scaffolds for structure activity relationship.

Susan Ramos-Hunter

G-protein activated inward rectifying potassium (K⁺) channels, or GIRKs are a part of a larger family of G-protein channel receptors that regulate potassium concentration in a cell. GIRK channels are found primarily in the brain as hetero and homotetramers and have demonstrated links to schizophrenia, epilepsy, pain perception and addiction and withdrawals. Research into GIRK channels has been limited but the key to understanding how these channels work has been instrumental with inhibitors and activator compounds. Recently, a high throughput screen has successfully isolated a GIRK activator, VU810 that was found to have an EC₅₀ of 160nM. Development of this scaffold has opened the door to further investigation of GIRK activators and inhibitors, leading to photo affinity labeling of a similar urea, 1-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-3-(3-(3-(trifluoromethyl)-3H-diazirin-3-yl)phenyl)urea. By complimenting the initial findings of the affinity for the GIRK binding pocket, the photo affinity tag will then act as a covalent bond, binding to the GIRK protein of interest and providing a clue to the structure activity relationship. This research offers key insights into a new territory of neurochemistry by the potential to understand GIRK targets and neuropathology.

Structure of Mitochondrial ADCK3 Defines an Ancient Protein Kinase-like Fold

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UbiB kinases are present in archaea, bacteria and eukaryotes, and comprise an estimated one-quarter of all microbial protein kinase-like (PKL) proteins. Structural studies have revealed the distinguishing features for most of the 20 known PKL families; however, members of the UbiB family have remained refractory to purification and biochemical analyses. UbiB kinases are associated with a diverse array of functions, most notably mitochondrial and plastid prenyl- and phospho-lipid biosynthesis, and are implicated in human cerebellar ataxia, nephrotic syndrome, and cancer. We report the first structure of a UbiB family member, human ADCK3, at 1.6 Å resolution. Our structure reveals that the unique N-terminal extension of the UbiB family does not form a separate accessory domain as in other kinases, but instead incorporates into the core kinase fold where it positions the invariant KxGQ motif into the nucleotide-binding pocket. Our analyses further define and validate the importance of additional UbiB-specific motifs, and provide insight into the molecular basis of a cerebellar ataxia caused by ADCK3 mutations. Collectively, our work defines an ancient and widespread variation on the PKL superfamily and provides a foundation for biochemical investigation and therapeutic targeting of the UbiB kinases.

Label-Free Assay for Classifying Intact Human Breast Cancer Cells Using Desorption Electrospray Ionization Mass Spectrometry

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Endogenous fatty acid synthesis is up-regulated in breast cancer. Phospholipids have been implicated in a number of cellular processes including signaling, proliferation, and apoptosis, but their role in cancer is largely unknown. In an effort to understand how lipids mediate tumor progression and oncogene expression, intact human various breast cancer cells having were interrogated by desorption electrospray ionization mass spectrometry imaging (DESI-MSI). This *in vitro* cell-based workflow provides rapid and detailed analysis with little sample preparation compared to traditional extraction and chromatography based methods. Principal component analyses of cell lipid profiles allowed identification of lipid features that correlated cell lines based on oncogene expression and breast cancer subtype. Breast cancer cell lines representing changes in metastatic potential and grade were also distinguished within a single assay. Tandem MS analyses identified of over 200 individual lipid species in negative ion mode, which is currently one of the most comprehensive phospholipid composition of breast cancer cells. These results demonstrate that DESI-MS based assays are powerful tools for rapidly classifying of cancer cells according to their geno- and phenotypes, while simultaneously providing extensive lipid compositions relevant to elucidating important biochemical mechanisms in cancer progression.

Small Molecule Inhibitors of the Ash1L Histone Methyltransferase

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Ash1L is an important epigenetic regulator and histone methyltransferase (HMTase) that activates *Hoxa9* and *Meis1* oncogenes.¹ During mouse embryonic stem cell differentiation, Ash1L's HMTase activity is required for expression of an additional 152 genes, including members of the *Hox* and *Wnt* families.² Ash1L is overexpressed in renal, breast, and skin cancer,³ but the role of Ash1L in oncogenesis is unclear at this time. Notably, Ash1L has also been implicated as a causative factor in facioscapulohumeral muscular dystrophy and liver fibrosis.^{4,5} We are developing small molecule inhibitors of Ash1L HMTase activity, which will be valuable chemical tools to assess the role of Ash1L's HMTase activity in a variety of disease systems. We are specifically interested in testing the hypothesis that Ash1L's HMTase activity is required for its activation of oncogenic *HOX* genes, and that small molecule inhibitors of Ash1L's HMTase activity may decrease *HOX* gene expression and slow cancer cell growth. To develop Ash1L inhibitors, we took a fragment-based screening approach, testing approximately 1500 small fragment-like molecules for binding to the Ash1L catalytic SET domain by NMR. We found 3 fragment-like ligands that bind to an allosteric site distinct from the S-adenosyl methionine (SAM) cofactor binding pocket. Medicinal chemistry optimization of one of the fragments led to improvement in affinity of more than 50-fold. We have also developed an enzyme assay for measuring the activity of our compounds and show that currently our most potent inhibitor has an IC₅₀ value of 22 μ M. We are actively working on co-crystallizing our compounds with Ash1L, which would provide structural information to further improve compound binding affinity. Once we develop compounds with low micromolar activity, we will begin testing these compounds for inhibition of histone methylation and *HOX* gene expression in human leukemic cells.

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Mutasynthesis of Apoptolidin Analogs

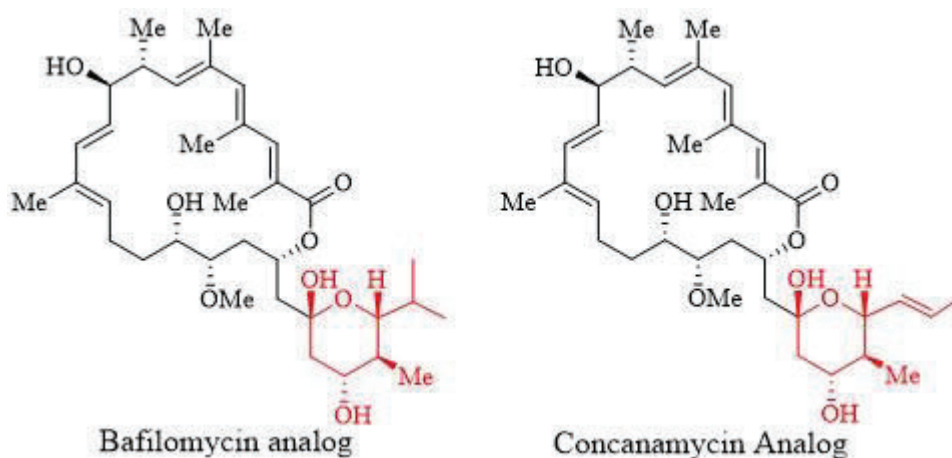
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Three polyketide ATPase inhibitors, apoptolidin, bafilomycin and concanamycin, show high structural homology in their large macrolactone cores and six-membered hemiketal rings; however, apoptolidin inhibits F-ATPase and the plecomacrolides, bafilomycin and concanamycin, inhibit V-ATPase.^{1,2} We have proposed an evolutionary relationship between both enzymes and their respective inhibitors and that the pyran moiety is the major determining factor in the selective inhibition of either F-ATPase or V-ATPase. To probe these hypotheses, two chimeric apoptolidin analogs (Figure 1) containing the macrolactone of apoptolidin and the pyran rings of bafilomycin and concanamycin respectively will be synthesized via mutasynthesis and total synthesis.

Figure 1



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‘Negotiations’ in Symbiosis: Periodic Provision of Host Chitin Drives Rhythms in Symbiont Metabolism

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Invertebrate models inform the study of the co-evolved dialogue that promotes cooperation between host and microbiota. For example, the bioluminescent bacterium *Vibrio fischeri* is maintained in the crypts of a specialized 'light organ' of the squid *Euprymna scolopes* throughout the squid's 6-month life. We hypothesize that the exchange of host nutrients for symbiont bioluminescence underlies the stability of the squid-vibrio mutualism.

In this study, we explored the contribution of the polysaccharide chitin to the host-symbiont 'negotiation'. Patterns of symbiont transcription indicate that chitin is present in the light organ only at night. Immunocytochemistry localized both proteins to macrophage-like blood cells called hemocytes, which contain granules of chitin. We also found that transcription of host chitin synthase and chitinase genes in light organs was subject to a diel rhythm that required tissue development and/or the presence of symbionts. Genetic and physiological studies revealed that symbiont catabolism of chitin produces acetate, and that this activity temporarily acidifies the crypts to a pH of ~5.5. Thus, the nightly provision of chitin by host hemocytes potentiates a symbiont-driven cycle of acidification in the light-organ crypt lumen.

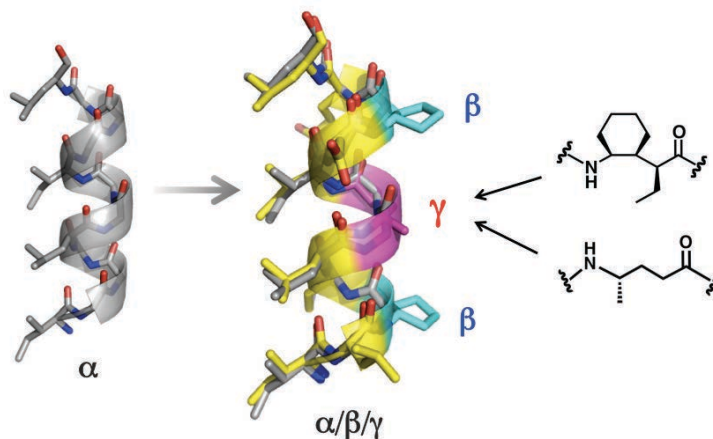
Bioluminescence is the central 'negotiating point' of the squid-vibrio symbiosis: light is required to maintain a persistent colonization, and the symbionts require oxygen to produce light. Bioluminescence is also subject to a daily rhythm, and is brightest at night. Our data suggest that the delivery of oxygen to the symbionts by the pH-responsive host protein hemocyanin is enhanced by the crypts' nightly acidification. Because symbiont bioluminescence, in turn, entrains circadian regulators in the light organ, we propose that the nocturnal fermentation of chitin by symbionts contributes to the maintenance of the stable, yet dynamic daily cycle of the squid-vibrio symbiosis.

Development of helical peptidomimetics: Heterogeneous peptide backbones with α - and preorganized β - and γ - amino acids

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Protein-protein interactions that regulate a variety of cellular processes involve helical interfaces. Effective manipulation of these interactions is of great interest for clinical purposes. We have reported that $\alpha/\beta/\gamma$ -peptides in an $\alpha\gamma\alpha\beta\alpha$ hexad pattern can mimic the structure of a helical α -peptide heptad repeat. An $\alpha/\beta/\gamma$ -peptide with ring constrained β - and γ -amino acids showed a very stable and rigid helical structure, possibly due to the helical backbone pre-organization provided by these ring constraints. Here, we focus on deepening our understanding of α -helix-like $\alpha/\beta/\gamma$ -peptides to enable development of $\alpha/\beta/\gamma$ -foldamers as useful biomimetic materials. Specific goals include 1) assessment of the effects of incorporating ring-constrained β - and γ -residues on helical $\alpha/\beta/\gamma$ -peptides, 2) quantitative thermodynamic analysis of $\alpha/\beta/\gamma$ -peptide helical stability, 3) exploration of $\alpha/\beta/\gamma$ -foldamers to modulate BH3 domain/Bcl-xL recognition.



Nitric Oxide Signaling through *S*-nitrosoglutathione Transnitrosation

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Nitric oxide (NO) synthesized by nitric oxide synthase (NOS) plays essential roles in cellular signaling. The best-described role for NO is activation of soluble guanylate cyclase leading to the production of cyclic GMP (cGMP). Mounting evidence has revealed the importance of a separate, cGMP-independent NO signaling pathway involving cysteine *S*-nitrosation. However, the cellular pathways that lead to selective *S*-nitrosation of only a subset of cellular cysteines remain largely unknown. Solution chemistry of *S*-nitrosation includes NO oxidation to N₂O₃ followed by reaction with thiolates, radical recombination of NO and thiyl radicals, and transition metal catalyzed pathways. Once formed, nitrosothiols can be transferred between small molecule or protein thiols through transnitrosation reactions. Selectivity may be conferred through colocalization with NOS isoforms, protein–protein interaction driven transnitrosation reactions, regulation of *S*-nitrosoglutathione (GSNO) levels, or directed denitrosation of protein nitrosothiols. Here, we provide evidence through detailed kinetic analyses that transnitrosation signaling pathways can be initiated through auto-*S*-nitrosation of NOS and subsequent transnitrosation of *S*-nitrosated NOS with glutathione to form GSNO. In cells, GSNO is thought to be the primary small-molecule nitrosating agent. We show that thioredoxin exhibits site-specific and redox-controlled transnitrosation by GSNO. However, the remaining targets of GSNO transnitrosation have yet to be fully identified and characterized. Therefore, we used a competitive activity-based profiling method to quantify the reactivity of GSNO against >1,200 cysteines in parallel in a human proteome. Using this approach, we identified a subset of cysteine residues that are sensitive to GSNO transnitrosation. *S*-nitrosation of one of the GSNO transnitrosation targets, 3-hydroxyacyl-CoA dehydrogenase type-2 (HADH2), inhibits the enzymatic activity of HADH2 and potentially fatty acid breakdown in the mitochondria. *S*-nitrosation of another target, cathepsin D (CTSD), affects the ability of CTSD to be proteolytically processed to its active form and potentially the involvement of CTSD in the pathogenesis of several diseases, including breast cancer and Alzheimer's disease.

Biomedical Applications and Strategies Utilizing Boronic Acid

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Boronic acids have been shown to interact with biologically relevant molecules including carbohydrates and reactive oxygen species (ROS). This variety of biological targets is due to the unique reactivity of boron that, within a physiological pH range, can adopt two electronically and structurally distinct forms that differ in Lewis acidity. We aim to exploit this reactivity in the design of novel biomedical tools and applications.

We are interested in exploring the reactivity of boron with biological hydroxyl groups for the development of small molecule tools for therapeutic delivery and protein stability. The development of therapeutic macromolecules is gaining considerable interest. Although there are intense efforts to facilitate therapeutic uptake into the cytosol, many of these strategies are limited by toxicity or stability. Our group, and others, have previously shown that boronic acids may offer a safe and effective method for macromolecule delivery via interactions with the diol-rich glycocalyx of the cell. We intend to expand upon these initial studies to develop a traceless strategy for cytosolic protein delivery.

Additionally, this reactivity can be probed further by designing small molecules that prevent protein misfolding. Familiar amyloid polyneuropathy (FAP) is a fatal genetic disease that is characterized by misfolding of the homotetramer protein transthyretin (TTR) leading to amyloid fibril formation in the peripheral nervous system. Preliminary results suggest that small boronic acid containing molecules can stabilize native TTR quaternary structure preventing amyloid fibril formation.

Development of Selective and Bioavailable Inhibitors of Collagen Prolyl 4-Hydroxylase

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Collagen is the most abundant protein in animals and serves as the principle component of bone, connective tissues, and the extracellular matrix. A variety of diseases are associated with the overproduction of collagen including the fibrotic diseases and cancer metastasis. The stability of collagen relies heavily on post-translational modifications that occur throughout the secretory pathway. Arguably the most important of these modifications is the hydroxylation of collagen strands by collagen prolyl 4-hydroxylase (CP4H), an Fe(II)- α -ketoglutarate (AKG)-dependent dioxygenase (FAKGD) located in the lumen of the endoplasmic reticulum. Catalysis by CP4H converts (2*S*)-proline residues in procollagen strands to (2*S*,4*R*)-4-hydroxyproline residues, which are essential for the conformational stability of mature collagen strands *in vivo*. Importantly, CP4H is a validated target for treatment of both the fibrotic diseases and metastatic breast cancer, which in aggregate represent a significant unmet clinical need. An effective therapy has yet to emerge from this strategy, due largely to the difficulty in designing selective inhibitors for enzymes of the FAKGD family. Herein, we report on the development of selective and bioavailable inhibitors of human CP4H. Using a chemical approach that takes advantage of newly developed methods in the palladium-catalyzed direct arylation of heterocycles, we redesign the classic 2,2'-bipyridine scaffold to generate potent biheteroaryl inhibitors of CP4H that are selective for enzymic iron. Further evaluation of these inhibitors in cell culture suggests that they are bioavailable and inhibit collagen synthesis at concentrations that do not disrupt iron homeostasis or cause general cytotoxicity. These results suggest the potential use of these inhibitors as leads for the development of antifibrotic and antimetastatic therapeutics.

This work was supported by grant R01 AR044276 (NIH).

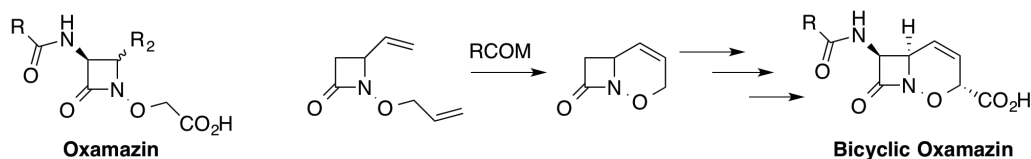
The Development of Metal-Mediated Methodologies for the Syntheses of Potential Antibiotics

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The discovery of penicillin in the early half of the 20th century represents a crucial point in the history of human healthcare and its introduction into widespread use induced a dramatic improvement in the quality of life and average life expectancy. While healthcare took a dramatic turn for the better with the advent of antibiotic therapy, the era of β -lactam antibiotics is also characterized by another important phenomena: the rise of bacterial resistance. The appearance of resistant strains of bacteria is a growing concern and, since the pipeline of available antibiotics has been diminished, the development of new drugs is becoming more and more urgent.

Oxamazins are a class of monocyclic heteroatom activated β -lactams that show good biological activity against Gram-negative bacteria. The presence of the oxygen atom directly bonded to the nitrogen is considered to be responsible for the electronic activation of the azetidinone ring and, therefore, the biological activity of these molecules. Our group and others have become interested in the syntheses of bicyclic oxamazins since the combination of the bicyclic structure and the heteroatom activation is envisaged to afford compounds with improved antibiotic activity.

We recently demonstrated that the core bicyclic oxamazin structure can be synthesized utilizing Grubbs' ring-closing olefin metathesis. This methodology also gave access to larger ring system bicyclic oxamazins. Our current efforts have focused on the incorporation of peripheral functionalization including the C-3 acylamino group and ionizable group – both thought necessary for antibiotic activity.



Investigation and Characterization of Scanning Ion Conductance Microscopy Resolution

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Scanned probe microscopy (SPM) resolution is a complex issue dependent upon factors such as probe size, probe-surface interaction, and sample geometry. Scanning ion conductance microscopy (SICM) is an SPM technique which uses a nanopipette probe to image nonconductive surfaces in a noncontact regime. SICM resolution has not been systematically investigated, but characterization of this technique's imaging capabilities has important implications for the quantitation of biological imaging. Previous studies performed with finite element method simulations have suggested that lateral resolution is limited to 2- 3x the size of the imaging probe; however, this estimation neglects a number of experimental parameters. To investigate technique resolution, a focused ion beam (FIB) was utilized to mill resolution standards with controlled dimensions onto a gold-coated glass coverslip. Lateral resolution below the size of the probe opening has been achieved, and has been shown to be dependent on a number of other factors. In the future, the characterization of SICM resolution will be applied to the quantitation of resolution of an advanced SICM technique, termed potentiometric-scanning ion conductance microscopy (P-SICM).

Small Molecule Modulation of RhlR Activity Induces Inverse Regulation of Pyocyanin and Rhamnolipid Production In *Pseudomonas aeruginosa*

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The gram-negative pathogen *Pseudomonas aeruginosa* uses an intercellular communication system known as quorum sensing (QS) to regulate the expression of virulence phenotypes. Together, the QS signal receptors LasR, RhlR, and PqsR form an interwoven regulatory network that exerts control over the global expression of numerous virulence-associated genes. Interception of these transcriptional regulators using small molecules represents a promising strategy for the development of anti-infective agents. However, a more thorough understanding of how modulation of specific QS circuits affects the expression of virulence phenotypes is needed before effective anti-virulence strategies can be developed. We sought to investigate how perturbation of LasR and RhlR by non-native *N*-acyl-L-homoserine lactones (AHLs) affects the expression of virulence phenotypes in *P. aeruginosa*. We evaluated a focused library of AHLs for their effects on the production of pyocyanin, a redox-active secondary metabolite, and the rhamnolipid, a biosurfactant, in wild type *P. aeruginosa*. We found that AHLs that strongly inhibited pyocyanin production were also potent RhlR agonists while pyocyanin inducers proved to be RhlR antagonists. Pyocyanin modulation was largely independent of the AHLs' effect on LasR. We speculate that AHLs' inhibitory effects on pyocyanin production are through down-regulation of the PQS system by RhlR. In good agreement with their respective activities against RhlR, AHLs exhibited inverse effects in a rhamnolipid assay relative to the pyocyanin assay. Thus, a RhlR agonist induces rhamnolipid production while simultaneously repressing pyocyanin and *vice versa*. These results highlight the complex regulation of QS-controlled phenotypes, and demonstrate that modulation of a single receptor in *P. aeruginosa* can have diverse effects on QS-regulated genes. They also suggest that targeting multiple QS response regulators may be an effective anti-virulence strategy.

Identification of Potential Circadian Rhythm Modulators

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The circadian clock is a cell-independent cycle that affects many important biological processes, including sleep-wake cycle and the release of hormones, and has been implicated in various metabolic diseases and sleep disorders. The role of the circadian rhythms in physiology is not well established so the discovery of small molecule modifiers to probe function in circadian disorders will provide a better understanding of circadian-associated diseases. To this end we have developed circadian rhythm cell-based assay that provided a luminescent readout. A high throughput screen identified a phenazine scaffold as a lead circadian rhythm period elongator and oxotetrahydroquinolinone scaffold as a lead period shortener. Amide analogs of these compounds will be screened for activity using the *rat1 Per2* promoter cell line and a luciferase expression reporter to determine their effects on the circadian rhythm period.

Recognition of Non-Mammalian Glycan Epitopes by Human Intelectin

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Differences in cell surface glycans can serve as markers of a cell's identity—its origin, its developmental state, or whether it is self- or non-self. Specific recognition of these surface glycoconjugates is paramount for biological processes ranging from reproduction to immune function. To discriminate self- from non-self cells, humans utilize proteinaceous carbohydrate-binding proteins, or lectins. For example, soluble lectins of the innate immune system are among the first host-derived biomolecules to recognize microbes. We found that human intelectin (hIntL) binds the non-human monosaccharide β -D-galactofuranose (Gal_f) with high affinity and selectivity. Intriguingly, glycan array screening technology revealed that hIntL fails to bind mammalian carbohydrate epitopes. To investigate this furanoside specificity, we are currently pursuing structural studies. Our results indicate hIntL is the first human lectin found to be specific for nonhuman glycan epitopes. Moreover, its ability to serve as a furanoside-selective lectin suggests it will be a valuable tool for detecting and diagnosing pathogens that display galactofuranose-containing glycans.

Modular Surfaces Deliver Insoluble Cues to Promote Mesendoderm Differentiation of Human Pluripotent Stem Cells

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Human pluripotent stem (hPS) cells possess the extraordinary capacity to self-renew indefinitely and differentiate into specialized cell types. Soluble growth factors and small molecules are powerful means of influencing hPS cell differentiation. Protocols for differentiation typically rely on these cues while employing substrata consisting of complex mixtures of biomolecules, such as Matrigel. Such surfaces interact with myriad cellular receptors, obscuring the influence of specific insoluble signals on hPS cell fate. We demonstrate that modular peptide-modified surfaces can deliver insoluble signals that help guide differentiation. Surfaces that specifically engage glycosaminoglycans are superior to Matrigel in promoting hPS cell differentiation to definitive endoderm, mesodermal progenitors or cardiomyocytes. The superiority of the modular surfaces rests in their ability to modulate signaling pathways. Specifically, we show integrins and integrin-linked kinase activate the Akt signaling pathway, which is antagonistic to mesendoderm differentiation. The ability to attribute cellular responses to specific interactions between the cell and the substrate can advance the development of strategies to optimally activate signaling pathways governing cell fate.

The Role of Substrate Elasticity in Influencing the Differentiation of Human Pluripotent Stem Cells to Neurons

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Human pluripotent stem (hPS) cells possess the remarkable capacity to self-renew indefinitely and differentiate into virtually all cell types. HPS cells thus represent an unlimited source of cells with potentially transformative applications such as modeling of human developmental and disease processes, cell-based regenerative medicine, and drug discovery and toxicity testing. All of these applications, however, require efficient and reproducible methods for propagating hPS cells and directing their differentiation to desired cell types. To date, focus has been on how soluble factors such as growth factors and small molecules influence these pathways. In physiological settings, however, stem cells receive both soluble and insoluble signals. Yet the role of insoluble signals, such as substrate elasticity, in influencing hPS cell fate decisions is less clear. We found that, even in the presence of soluble factors that promote pluripotency, compliant substrata, with elasticity similar to human brain tissue, override these signals to induce efficient differentiation of hPS cells to neurons. The neurons derived by substrate induction alone—in absence of neurogenic factors—express mature markers and possess action potentials. The molecular mechanism is through modulation of the subcellular localization of the transcriptional coactivator YAP. Nuclear exclusion of YAP in cells cultured on compliant substrata or the depletion of YAP by RNA interference effects neuronal differentiation. Our findings indicate that mechanical cues can override soluble signals, suggesting that their contributions to early human development and *in vitro* differentiation are profound. Current hPS cell differentiation protocols are almost exclusively carried out on tissue culture polystyrene, a substrate which is orders of magnitude stiffer than biological tissues. Therefore, we anticipate that utilizing substrates with more biologically relevant mechanical properties will increase the efficiency of existing differentiation protocols and perhaps give access to currently elusive cell types.

Simultaneous Site-specific Dual Protein Labeling Using Multiple Protein Prenyltransferases

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Protein prenylation is a post-translational modification that involves the attachment of isoprenoid groups to proteins. It has been widely explored as a method for site-specific protein labeling¹. By incorporating different functional groups into the target protein, this labeling strategy can be applied to protein immobilization², protein PEGylation³, proteomic analysis⁴, etc. Here, we developed a new labeling strategy to simultaneously label multiple proteins. In this approach, an alkyne containing isoprenoid analogue can be recognized by PFTase and incorporated into a target protein with a C-terminal CVIA sequence while a ketone containing isoprenoid analogue can be incorporated by GGTase-I and appended to proteins with a C-terminal CVLL sequence. We showed that these two reactions were highly specific and when performed together, no side product from cross-reaction was produced. The labeled proteins were subsequently linked to fluorophores via a combination of oxime formation and “click” reaction. Thus, this strategy extends the utility of protein labeling using prenyltransferases and shows great potential for pulling down multiple proteins simultaneously and creating patterned protein microarrays.

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Synthetic antigens and calcium flux imaging reveal mechanisms of B cell-T cell cooperation

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B cells respond to specific pathogen-derived antigens via B cell antigen receptors (BCRs). An effective immune response to pathogens depends on B cell-T cell interactions that occur upon BCR-mediated internalization, processing and presentation of foreign antigens to CD4⁺ T cells. Antigens vary widely in structural properties such as valency. Yet the influence of these features on antigen presentation and T cell responses remains unclear. We have developed chemically synthesized antigens that vary systematically in structure to investigate this question. Utilizing these synthetic antigens, we provide insights into mechanisms of antigen uptake, processing and presentation to T cells. Furthermore, we employ calcium flux imaging in individual T cells to reveal how antigen structure influences mechanisms of T cell activation. These findings demonstrate that defined synthetic antigens can reveal how diverse structural properties influence T cell responses.

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